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The use of a tannin crude extract from *Cistus ladanifer* L. to protect soya-bean protein from degradation in the rumen

M. T. P. Dentinho†, O. C. Moreira, M. S. Pereira and R. J. B. Bessa

Estação Zootécnica Nacional, Fonte Boa, 2005-048 Vale de Santarém, Portugal

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Cistus ladanifer L. (CL) is a perennial shrub abundant in dry woods and dry land of Mediterranean zone, with high level of tannins. Tannins bind to protein, preventing its degradation in the digestive compartments. This tannin/protein complex may be advantageous when partially protecting good-quality feed protein from excessive rumen protein degradation. The objective of this trial was to use a CL phenol crude extract to prevent excessive rumen degradation of soya-bean meal protein. The phenolic compounds were extracted using an acetone/water solution (70:30, v/v). Soya-bean meal was then treated with this crude CL extract, containing 640 g of total phenols (TP) per kg of dry matter (DM), in order to obtain mixtures with 0, 12.5, 25, 50, 100 and 150 g of TP per kg DM. Three rumen-cannulated rams were used to assess in sacco rumen degradability of DM and nitrogen (N). The three-step in vitro procedure was used to determine intestinal digestibility. Increasing extract concentrations quadratically decreased the N-soluble fraction a ($R^2 = 0.96$, $P = 0.0001$) and increased the non-soluble degradable fraction b ($R^2 = 0.92$, $P = 0.005$). The rate of degradation c linearly decreased with CL extract doses ($R^2 = 0.44$, $P = 0.0065$). For the effective rumen degradability of N, a linear reduction ($R^2 = 0.94$, $P < 0.0001$) was observed. The in vitro intestinal digestibility of protein (ivID) quadratically decreased ($R^2 = 0.99$, $P < 0.0001$) with TP inclusion and the rumen undegradable protein (RUP) showed a quadratic increase ($R^2 = 0.94$, $P = 0.0417$). Total intestinal protein availability, computed from the RUP and ivID, linearly decreased with TP inclusion level ($R^2 = 0.45$, $P = 0.0033$).

Keywords: Cistus ladanifer *L, intestinal digestibility, rumen undegradable protein, soya-bean meal, tannins*

Introduction

Tannins are phenolic secondary compounds of plants and are usually classified into two groups based on their chemical structures: hydrolysable and condensed tannins (Min *et al.*, 2003). Hydrolysable tannins contain a carbohydrate core (often glucose) esterified with gallic acid or ellagic acid. Condensed tannins (CT) are the most common tannin type found in forage legumes, trees and shrubs (Barry and Mcnabb, 1999) and are oligomers or polymers of flavanoid units linked by carbon-carbon bonds (Hagerman, 1988) CT can complex with numerous types of molecules including proteins, polysaccharides, and minerals (McSweeney *et al.*, 2001). The multiple phenolic hydroxyl groups of CT lead to the formation of complexes primarily with proteins and to a lesser extent with polysaccharides (Makkar, 2003).

CT have both adverse and beneficial nutritional effects in herbivores depending on their chemical structure and dietary concentration (Makkar, 2003; Min *et al.*, 2003). Adverse effects of tannins include lower intake and digestibility of protein and carbohydrates, inhibition of digestive enzymes and lower animal performance (Butter *et al.*, 1999; Getachew *et al.*, 2000). The beneficial effects of CT are associated with their capacity to prevent bloat, increase digestive utilisation of dietary protein for ruminants and act as anthelmintics and antioxidants (Mueller-Harvey, 1999; Makkar, 2003).

The potential of CT to increase the digestive utilisation of dietary protein for ruminants is associated to their ability to bind proteins under the rumen pH conditions (pH 5.5 to 7.0), preventing the excessive microbial degradation of proteins. The tannin-protein complexes are dissociated in the acidic pH of the abomasum (pH 2.5 to 3.5) and in alkaline conditions of the distal small intestine (pH $\approx$7.5) releasing protein for digestion and absorption (Barry *et al.*, 1986).

The use of CT, as feed additives, to improve the digestive utilisation of dietary protein in ruminants has been successfully explored by several authors. Salawu *et al.* (1999) used three commercial tannin sources (quebracho, mimosa and

† E-mail: ezn.inia@mail.telepac.pt

myrabolam) as silage additives and Frutos *et al.* (2000) used commercial quebracho tannin extracts as additive for protecting soya-bean meal protein against rumen degradation. Tannins extracted from *Cistus ladanifer* L. (CL) reduce *in sacco* rumen protein degradability (Dentinho *et al.*, 2000). CL is a very abundant shrub in marginal fields of Mediterranean countries. It contains a high level of CT (Dentinho *et al.*, 2000) and is practically not used in direct grazing due to its low nutritive value (Rodriguez *et al.*, 1989). In the present study, we explored the utilisation of a phenolic extract from CL to reduce rumen degradation of soya-bean meal protein.

Material and methods

Preparation of phenolic extract

Phenolic compounds were extracted from CL (leaves and soft stems) harvested in March 2002 in the Southwest of Portugal. CL samples were freeze-dried and ground to pass through a 3-mm screen. Nine portions of 75 g each were weighed into 1-l round bottom flasks. To remove fats and pigments, 300 ml of petroleum ether was added to each sample, which was then stirred (KS 260, IKA®-Werke, Germany) for 30 min (Terril *et al.*, 1990). This washing procedure was repeated twice. The supernatant was discarded and the residue was extracted three times with an acetone: water solution (7:3, v/v) (adapted from Hagerman (1988)). The first extraction was made with 750 ml on an orbital shaker (KS 260, IKA®-Werke, Germany) for 2 h, and the other two extractions with 250 ml, for 1 h. The supernatant containing the phenolic compounds was pooled and the acetone was removed by rotary evaporation at 30 °C. This extract was then stored at − 20 °C and freeze-dried.

Preparation of soya-bean meal products

Six samples of 500 g of soya-bean meal (solvent extracted and 440 g/kg of crude protein (CP) were weighed into plastic bags and sprayed using a compressor (Model Montecarlo OL 231, ABAC, UK), with 300 ml of CL phenolic extract dissolved in acetone:water (70:30 v/v). The extract was added in the following doses: 0, 9.8, 19.5, 39.0, 78.0 and 117 g aiming at obtaining soya-bean meals products with 0 (S0), 12.5 (S12.5), 25 (S25), 50 (S50), 100 (S100) and 150 (S150) g TP per kg. The samples were air-dried for 24 h to remove acetone and then oven-dried at 40 °C for 24 h.

Rumen in sacco degradability

Nylon bags from nitrogen free polyester with pore size of 50 μm diameter (50 mm × 100 mm; ANKOM Technology, Spain) were filled with 5 g of one of the six soya-bean products. Six bags, each containing one of the soya-bean meal products, were simultaneously incubated in the rumens of three cannulated rams, before the morning feeding for 2, 4, 6, 8, 16, 24, 48 or 72 h. In order to be technically feasible, the eight different incubation periods were started on separate days. Experimental conditions, including feeding management, was standardised during the whole trial. Animals were fed lucerne hay (153 g CP per kg of dry matter (DM) and 536 g neutral-detergent fibre (NDF) per kg DM) and a commercial concentrate (Table 1) in a proportion of 60/40 (w/w) at maintenance (50 g DM per kg $M^{0.75}$ per day). Rams were fed twice daily (0930 and 1730 h) two equal portions. After incubation, the bags were washed twice in a washing machine (Model AWG 652 Whirlpool, USA), with cold water during 20 min and dried to constant weight at 45 °C in a forced air oven. Zero-time losses were estimated by washing in the same washing machine (20 min), three bags per sample without previous rumen incubation. The disappearance values of DM and nitrogen (N) were fitted to the Ørskov and McDonald (1979) model, $p = a + b\ (1\text{-}e^{-ct})$ where a represents the soluble or rapidly degradable fraction, b represents the non-soluble degradable fraction which disappears at a constant fractional rate c per unit of time and where $a + b \leq 1$. The $P = a + [bc/(c + k)]$ equation was used to estimate effective degradability (P) where k (the outflow rate from the rumen) was assumed to be 0.08 per h (Ørskov and McDonald, 1979).

Intestinal in vitro *digestibility*

Intestinal protein digestibility (ivID) was determined by the three-step *in vitro* procedure developed by Calsamiglia and Stern (1995). In this method, one nylon bag for each soya-bean meal product was suspended in the rumen of three cannulated rams for 16 h. This process was repeated three times. The residues were washed and dried to constant weight at 45 °C. For each animal, residues of the three bags were pooled to ensure sufficient soya-bean meal residue for further incubation and added to form a composite sample. The composite samples were analysed for N using a Kjeldhal method (International Organization for Standardization (ISO), 1997). The intestinal digestion was simulated on pooled sample residues. Sample residues containing 15 mg of N were incubated for 1 h in 10 ml of 0.1 mol/l HCl solution containing 1 g/l of pepsin (Sigma P-7012;

Table 1 *Composition of the concentrate fed to the three rumen cannulated sheep used for the in sacco experiment*

Ingredient	g/kg dry matter
Wheat	228
Barley	250
Maize gluten feed	270
Sunflower meal	150
Soya-bean meal	60
Calcium carbonate	25
Dicalcium phosphate	5
Vitamin and trace mineral pre-mix[†]	2
Salt	10
Chemical composition	
CP	189
NDF	203

[†] Provided per kg: retinol 1500 mg; cholecalciferol 27.5 mg; alpha-tocopherol 2700 mg; Mg 25 g; Fe 15 g; Zn 25 g; Mn 15 g; I 500 mg; Se 50 mg; Co 250 mg.

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Sigma, St Louis, USA), after which pH was neutralised with 0.5 ml of 1 mol/l NaOH. Afterwards, 13.5 ml of a pH 7.8 phosphate buffer containing 37.5 mg of pancreatin (Sigma P-7545) were added to the solution and incubated at 38 °C for 24 h. Then, 3 ml of a 100% (w/v) trichloroacetic acid (TCA) solution was added to stop enzymatic action and precipitate undigested proteins. The samples were centrifuged at 10 000 **g** for 15 min and the supernatant was analysed for soluble nitrogen (sol-N). Pepsin-pancreatin digestion of protein was calculated as TCA – soluble N divided by the amount of sample N (nylon bag residue) used in the assay. The incubation procedure was performed twice.

Chemical analyses

CL phenolic extract was analysed in duplicate for DM (ISO, 1999), N using the Kjeldhal method (ISO 5983, 1997) and total sugar (Clegg, 1956).

Extraction and analysis of phenolic compounds were carried out in four replicates as described by Khazaal *et al.* (1993). Samples (200 mg) of CL were extracted by ultrasonication (Model 200 TH/2l, VWR International, Lisboa, Portugal) using 10 ml of 70% aqueous acetone in an ice bath for 10 min. The extract obtained was centrifuged at 1400 **g** at 4 °C for 30 min and the supernatant was used as 'original extract' for TP and CT assays. TP were determined by Folin-Ciocalteu's reagents, according to Julkunen-Tiito (1985) and the concentration was measured as tannic acid equivalent using tannic acid (100 773, Merck KGaA, Darmstadt, Germany) as standard.

Total extractable CT were measured using the vanillin assay (CTv) of Broadhurst and Jones (1978). CTv were expressed as catechin equivalent using catechin (Sigma C-1788) as standard. Total tannins were measured by a protein precipitation assay, the radial diffusion method (TTdr), performed in agarose plates with a protein, the bovine serum albumin (Sigma A-7906) (Hagerman, 1987).

Soya-bean meal samples were analysed for DM (ISO, 1999), N (ISO, 1997), total sugar (Clegg, 1956) and for sol-N by solubilisation in artificial saliva (Dulphy and Demarquilly, 1981) and for NDF and acid-detergent fibre by the methods of Van Soest *et al.* (1991). NDF was assayed with sodium sulphite, without alpha amylase and expressed with residual ash. The *in vitro* organic matter digestibility (OMD) was determined by the Tilley and Terry method modified by Alexander and McGowan (1966). Bag residues of the *in sacco* degradability trial were analysed for N (ISO, 1997)

Statistical analysis

The general linear model (GLM) procedure (Statistical Analysis Systems Institute, 2004) was used to regress the changes in chemical composition (total N, sol-N, sugar, NDF, ADF and OMD), rumen degradation parameters (*a*, *b*, *c*) of DM and CP, P, rumen undegradable protein (RUP), ivID and total protein availability (TPA) according to the inclusion level of TP. The model used was:

$$Y_i = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_i$$

where Y_i is the value of studied parameters, β_0 the mean response of *Y*when $X = 0$, β_1 is the linear effect coefficient, β_2 is the quadratic effect coefficient, X_i the value of the predictor variable, ε_i is the random error.

Results

The CL extract obtained was a crude extract that contained sugar (163 g/kg DM), N (3.5 g/kg DM) and high levels of TP, condensed and total tannins (Table 2).

The chemical composition of soya-bean meal products is presented in Table 3. Increasing concentrations of phenols linearly decreased total N ($R^2 = 0.93$, $P < 0.001$) and OMD ($R^2 = 0.96$, $P < 0.01$) and linearly increased NDF ($R^2 = 0.83$, $P < 0.001$). For sol-N a quadratic decrease with phenolics concentration ($R^2 = 0.97$, $P < 0.001$) was observed.

Table 4 shows *in sacco* DM and N degradation parameters and effective degradability (P), computed assuming a ruminal passage rate of 0.08 per h, of soya-bean meal treated with different doses of CL extract. A quadratic effect of the CL extract doses was observed leading to a decrease in fraction *a* ($R^2 = 0.96$, $P = 0.0001$) and an increase in fraction *b* of N ($R^2 = 0.92$, $P = 0.005$). The CL extract linearly decreased the DM *a* fraction ($R^2 = 0.80$, $P < 0.0001$), whereas *b* was linearly increased ($R^2 = 0.66$, $P < 0.001$). Consequently, the potential degradability ($a + b$) remained unchanged (data not showed). The rate of degradation *c* of DM and N linearly decreased with the inclusion of CL extract (respectively $R^2 = 0.59$, $P = 0.0005$ and $R^2 = 0.44$, $P = 0.0065$). The P of DM and N of treated soya-bean meals linearly decreased at higher CL extract levels (respectively $R^2 = 0.85$ $P < 0.0001$; $R^2 = 0.94$ $P < 0.0001$) mainly due to a reduction of the soluble or rapidly degradable fraction *a*. The effect was greater in N than in DM degradability.

The ivID, RUP and TPA are presented in Table 5. Soya-bean meal protein without tannins had a low ivID (0.61) and quadratically decreased with TP ($R^2 = 0.99$, $P < 0.0001$). The RUP quadratically increased with the TP

Table 2 *Chemical composition of phenolic crude extract of* Cistus ladanifer *L*

	C. ladanifer L. extract
Dry matter (g/kg)	910
Nitrogen (g/kg DM)	3.50
Sugar (g/kg DM)	163
Total phenolics[†]	640
Condensed tannins (vanillin)[‡]	315
Total tannins (radial diffusion)[†]	300

[†] Tannic acid equivalent in g/kg dry matter (DM).
[‡] Catechin equivalent in g/kg DM.

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Table 3 *Chemical composition and* in vitro *organic matter digestibility (OMD) (g/kg DM) of soya-bean meal treated with different doses of a polyphenolic extract of* Cistus ladanifer *L. (n = 2)*[†]

								P value	
	S0	S12.5	S25	S50	S100	S150	R^2	L	Q
N	80.7	77.9	78.8	75.2	72.1	69.2	0.93	<0.001	0.26
Sol-N	185	149	115	787	492	353	0.97	<0.001	<0.001
Sug	144	143	144	144	139	135	0.23	0.14	0.70
NDF	112	123	133	129	144	177	0.83	<0.001	0.05
ADF	81.0	87.6	82.2	84.5	78.1	93.8	0.52	0.14	0.70
OMD	790	810	794	767	708	665	0.96	<0.01	0.57

[†] Abbreviations are: N = nitrogen (g/kg DM), Sol-N: soluble N (g/kg of total- N), SUG = sugar (g/kg DM), NDF = neutral-detergent fibre (g/kg DM), ADF = acid-detergent fibre (g/kg DM), OMD = organic matter digestibility. S0, S12.5, S25, S50, S100, S150: soya-bean meal treated with total phenol concentrations (g/kg) of 0, 12.5, 25, 50, 100 and 150, respectively.

inclusion level ($R^2 = 0.94$, $P = 0.0417$). The TPA was computed from the RUP and ivID and linearly decreased with the inclusion of TP ($R^2 = 0.45$, $P = 0.0033$).

Discussion

Total phenols and tannins concentrations in crude extract were lower than those observed in commercial quebracho extract (Sarl André Hiriar, France) that was also analysed in our laboratory (770 g TP per kg DM, and 330 g TTdr per kg DM). However, CT determined by the vanillin method were lower in quebracho than those observed in the CL extract (315 *v.* 950 g/kg DM). The values obtained by radial diffusion for both CL extract and commercial quebracho were similar.

The decrease of total N with the increase of phenolic concentration in soya-bean meal was due to a dilution effect of the CL extract addition. The linear reduction of OMD and the quadratic reduction observed for sol-N, cannot completely be attributed to a dilution effect but, presumably, is also the result of the soluble proteins binding with CT. The NDF fraction in soya-bean meal increased with the CL extract inclusion probably because of the formation of 'artefact neutral fibre' as a result of fibre-tannin interactions. Formation of tannin complexes with protein and fibre components remain in the NDF and ADF fraction, thereby increasing the apparent lignin concentration, as reported by Carre and Brillouet (1986) and Van Soest *et al.* (1987). However, we did not observe any increase in ADF proportion with the incorporation of CL extract, which may suggest that the formed complexes may be soluble in acid-detergent solution but stable in neutral-detergent solution.

The CL extract had a depressive effect on rumen effective degradability of both DM and N of soya-bean meal. However, this effect is greater in N than in DM degradability, probably due to the particular affinity of CT for proteins (Makkar, 2003). The soluble or rapidly degradable fraction *a* of DM and N from soya-bean meal without tannins was much higher than that reported by Frutos *et al.* (2000). In the current study, this fraction may have been overestimated because no correction was made for the small particles washed out from the bags (mechanical losses). Nevertheless, the great negative relationship between total phenolic concentrations and fraction *a* of DM and N is evident and suggests a physical binding of CT with soluble proteins and carbohydrates.

Table 4 In sacco *soluble or rapidly degradable fraction* (a), *non-soluble degradable fraction* (b) *and fractional degradation rate of the* b *fraction* (c) *(per h) (Ørskov and McDonald, 1979) and effective degradability (P) of dry matter (DM) and nitrogen (N) of soya-bean meal treated with different doses of a polyphenolic extract of* Cistus ladanifer *L (n = 3)*[†]

								P value	
	S0	S12.5	S25	S50	S100	S150	R^2	L	Q
DM									
a	0.54	0.49	0.45	0.47	0.45	0.39	0.80	<0.0001	0.59
b	0.46	0.50	0.55	0.53	0.54	0.60	0.66	<0.0001	0.66
c	0.037	0.043	0.035	0.036	0.035	0.022	0.59	0.0005	0.17
P	0.69	0.66	0.62	0.63	0.61	0.52	0.85	<0.0001	0.27
N									
a	0.55	0.44	0.41	0.32	0.27	0.20	0.96	<0.0001	0.0001
b	0.45	0.56	0.59	0.68	0.71	0.80	0.92	<0.0001	0.0052
c	0.037	0.039	0.033	0.037	0.034	0.025	0.44	0.0065	0.19
P	0.69	0.63	0.59	0.54	0.48	0.40	0.94	<0.0001	0.04

[†] S0, S12.5, S25, S50, S100, S150: soya-bean meal treated with total phenol concentrations (g/kg) of 0, 12.5, 25, 50, 100 and 150, respectively. P: effective rumen degradability of DM and N calculated with outflow rate $k = 0.08$ per h.

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Table 5 *Intestinal digestibility of protein (relative to apparent rumen undegradable protein) (ivID), rumen undegradable (RUP) and total availability of protein (TPA) of soya-bean meal treated with different doses of a polyphenolic extract of* Cistus ladanifer *L. (n = 3)*[†]

								P value	
Protein	S0	S12.5	S25	S50	S100	S150	R^2	L	Q
ivID	0.61	0.56	0.52	0.46	0.40	0.39	0.99	< .0001	< .0001
RUP	0.31	0.37	0.41	0.46	0.52	0.60	0.94	< .0001	0.04
TPA[‡]	0.19	0.21	0.21	0.21	0.21	0.24	0.45	0.003	0.77

[†] S0, S12.5, S25, S50, S100, S150: soya-bean meal treated with total phenol concentrations (g/kg) of 0, 12.5, 25, 50, 100 and 150, respectively.
[‡] Rumen undegradable protein × protein intestinal digestibility.

The fractional degradation rate *c* of DM and N linearly decreased with the level of CL extract. These results are consistent with those obtained by Frutos *et al.* (2000) who found a depression in the *in sacco* degradation rate of soya-bean meal treated with 150 and 250 g/kg of quebracho tannins. Reduction of *in sacco* degradation rates of feeds induced by the presence of CT have been reported in other studies (Aharoni *et al.*, 1998; Min *et al.*, 2003). The depression in the degradation rate has been related either to the reduction in the attachment of microbes to feed particles (Makkar *et al.*, 1988; McAllister *et al.*, 1994) or to a specific inhibition of microbial growth and enzyme activity (McSweeney *et al.*, 2001). Still, the *in sacco* results should be interpreted with caution as pointed out by Khazaal *et al.* (1993). This technique may not be suitable for evaluating feeds with anti-nutritive effects because only the physical binding of polyphenols could be detected in a nylon bag incubated in a large environment (rumen), whereas other effects such as toxicity to microbes or binding to their enzymes would be diluted.

The ivID of soya-bean meal protein without tannins was very low (0.61). Using the same technique, Calsamiglia and Stern (1995) and Frutos *et al.* (2000) reported ivID for soya-bean meal protein of 0.89 and 0.94, respectively. Predicting intestinal digestibility in function of RUP and acid-detergent insoluble N (Agricultural and Food Research Council, 1993) the value obtained is 0.85. The ivID values of our current study remain considerably lower and reasons for such low values are unclear. Nevertheless, the increasing level of CL extract linearly decreased the protein ivID.

When using soya-bean meal treated with 10 to 250 g of quebracho tannins per kg, Frutos *et al.* (2000) observed a reduction in ivID for the highest level only, whereas protein ivID was depressed even at the lower phenolic doses in our study (12.5, 25 and 50 g/kg). Differences in intestinal digestibility of soya-bean meal treated with CL extract and soya-bean meal treated with quebracho may be associated with differences in the chemical structure of CT, as it determines the biological effects of tannins (Min *et al.*, 2003). Based on differences in tannin ability to bind protein in the rumen, Perez Maldonado *et al.* (1996) suggested that the post-ruminal reversibility of the process may also differ between tannins. Although, we did not find any differences in the radial diffusion test between quebracho and CL tannins, CL tannins probably formed protein/tannin complexes which are more stable at low pH. Alternatively, in this *in vitro* system, CL tannins dissociated at pH 1.9 could have higher affinity than quebracho tannins to bind pepsin and, after neutralisation at pH 7.8, to bind prancreatin. In fact, several studies suggest that the inhibition of digestive enzymes by dissociated tannins may occur (Makkar *et al.*, 1988; McSweeney *et al.*, 2001; Silanikove *et al.*, 1994).

TPA linearly increased with the inclusion of TP. Although, these values suffer from the oversimplification, which is intrinsic to *in sacco* and *in vitro* methods, the results suggest that between 12.5 and 100 g/kg of TP inclusion in soya-bean meal, the desirable rumen effects counterbalance the negative post-ruminal effects.

Conclusions

From this study we conclude that CL phenolic extract causes a reduction in rumen degradation of soya-bean meal protein, thus increasing the flux of potential feed protein into the post-ruminal compartments. However, the phenolic extract has a negative effect on ivID. Nevertheless, the estimated total availability of protein increased even with lower levels of CL extract inclusion. Nevertheless, animal studies are required to evaluate whether the reduction in rumen degradable protein does not limit microbial protein synthesis.

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References

Agricultural and Food Research Council 1993. Energy and protein requirements of ruminants. CAB International, Wallingford, UK.

Aharoni Y, Gilboa N and Silanikove N 1998. Models of suppressive effect of tannins. Analysis of the suppressive effect of tannins on ruminal degradation by compartmental models. Animal Feed Science and Technology 71, 251-267.

Alexander RH and McGowan M 1966. A filtration procedure for the *in vitro* determination of digestibility of herbage. Journal of the British Grassland Society 16, 140-147.

Barry TN, Manley TR and Duncan SJ 1986. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 4. Site of carbohydrate and protein digestion as influenced by dietary reactive tannin concentrations. British Journal of Nutrition 55, 123-137.

https://doi.org/10.1017/S1751731107689745 Published online by Cambridge University Press

Barry TN and Mcnabb WC 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. A review. The British Journal of Nutrition 81, 263-272.

Broadhurst RB and Jones WT 1978. Analysis of condensed tannins using acidified vanillin. Journal of the Science of Food and Agriculture 29, 788-794.

Butter NL, Dawson JM and Buttery PJ 1999. Effects of dietary tannins on ruminants. In Secondary plant products: antinutritional and beneficial actions in animal feeding (ed. JC Caygill and I Mueller-Harvey), pp. 51-70, Nottingham University Press, Nottingham, UK.

Calsamiglia S and Stern MD 1995. A three-step *in vitro* procedure for estimating intestinal digestion of protein in ruminants. Journal of Animal Science 73, 1459-1465.

Carre B and Brillouet JM 1986. Yield and composition of cell-wall residues isolated from various feedstuffs used for non ruminant farm animals. Journal of the Science of Food and Agriculture 37, 341-351.

Clegg KM 1956. The application of the anthrone reagent to the estimation of starch in cereals. Journal of the Science of Food and Agriculture 7, 40-44.

Dentinho MTP, Melo MM, Bessa RJB and Ribeiro JMR 2000. Efeito do polietilenoglicol sobre a degradabilidade ruminal da esteva (*Cistus ladanifer* L.). Proceedings of the X Congresso de Zootecnia, Vale de Santarém, Portugal, p. 74.

Dulphy JP and Demarquilly C 1981. Problèmes particuliers aux ensilages. In Prévision de la valeur nutritive des aliments des ruminants (ed. INRA Publ.), pp. 81-104, Versailles, France.

Frutos P, Hervás G, Giráldez FJ, Fernández M and Mantecón AR 2000. Digestive utilization of quebracho-treated soya bean meal in sheep. Journal of Agriculture Science, Cambridge 134, 101-108.

Getachew G, Makkar HPS and Becker K 2000. Effect of polyethylene glycol on *in vitro* degradability of nitrogen and microbial protein synthesis from tannin-rich browse and herbaceous legumes. British Journal of Nutrition 84, 73-83.

Hagerman A 1987. Radial diffusion method for determining tannin in plant extract. Journal of Chemical Ecology 13, 437-449.

Hagerman AE 1988. Extraction of tannin from fresh and preserved leaves. Journal of Chemical Ecology 14, 453-462.

International Organization for Standardization 1997. Animal feeding stuffs- determination of nitrogen content and calculation of crude protein content- Kjeldhal method, ISO 5983.

International Organization for Standardization 1999. Animal feeding stuffs-determination of moisture and other volatile matter content. ISO 6496.

Julkunen-Tiitto R 1985. Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics. Journal of Agricultural and Food Chemistry 33, 213-217.

Khazaal K, Markantonatos X, Nastis A and Ørskov ER 1993. Changes with maturity in fiber composition and levels of extractable polyphenols in greek browse: effects on *in vitro* gas-production and *in sacco* dry matter degradation. Journal of the Science of Food and Agriculture 63, 237-244.

McAllister TA, Bae HD, Jones GA and Cheng KJ 1994. Microbial attachment and feed digestion in the rumen. Journal of Animal Science 72, 3004-3018.

McSweeney CS, Palmer B, McNeil D and Mand Krause DO 2001. Microbial interactions with tannins: nutritional consequences for ruminants. Animal Feed Science and Technology 91, 83-93.

Makkar HPS 2003. Effects and fate of tannins in ruminant animals, adaptation to tannins, and strategies to overcome detrimental effects of feeding tannin-rich feeds. Small Ruminant Research 49, 241-256.

Makkar HPS, Singh B and Dawra RK 1988. Effect of tannin-rich leaves of oak (Quercus Incana) on various microbial enzyme-activities of the bovine rumen. British Journal of Nutrition 60, 287-296.

Min BR, Barry TN, Attwood GT and McNabb WC 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Animal Feed Science and Technology 106, 3-19.

Mueller-Harvey I 1999. Tannins their nature and biological significance. In Secondary plant products: antinutritional and beneficial actions in animal feeding (ed. JC Caygill and I Mueller-Harvey), pp. 17-39, Nottingham University Press, Nottingham, UK.

Ørskov ER and McDonald I 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. Journal of Agricultural Science, Cambridge 92, 499-503.

Perez-Maldonado RA and Norton BW 1996. The effects of condensed tannins from *Desmodium intortum and Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. British Journal of Nutrition 76, 515-533.

Rodriguez MS, Castro AGG, Lucena EP, Moreno CM and Leal JLA 1989. Papel de los Cistus en el pastoreo caprino. Proceedings of the II Reunião Ibérica de Pastagens e Forragens, Elvas, Portugal.

Salawu MB, Acamovic T, Stewart CS, Hvelplund T and Weisbjerg MR 1999. The use of tannins as silage additives: effects on silage composition and mobile bag disappearance of dry matter and protein. Animal Feed Science and Technology 82, 243-259.

Statistical Analysis Systems Institute 2004. SAS/STAT 9.1 user's guide. SAS Inst. Inc., Cary, NC, USA.

Silanikove N, Nitsan Z and Perevolotsky A 1994. Effect of a daily supplementation of polyethylene glycol on intake and digestion of tannin-containing leaves (Ceratonia siliqua) by sheep. Journal of Agricultural and Food Chemistry 42, 2844-2847.

Terrill TH, Windham WR, Evans JJ and Hoveland CS 1990. Condensed tannin concentration in *Sericea lespedeza* as influenced by preservation method. Crop Science 30, 219-224.

Van Soest PJ, Conklin NL and Horvath PJ 1987. Tannins in foods and feeds. In Proceedings of the Cornell nutrition conference for feed manufacturers. Cornell University, Ithaca, NY.

Van Soest PJ, Robertson JB and Lewis BA 1991. Methods for dietary fibre, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science 74, 3583-3597.