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RESEARCH ARTICLE

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The use of polyethylene glycol to reduce the anti-nutritional effects of tannins in *Cistus ladanifer* L.

Maria Teresa P. Dentinho[1,2], Olga C. Moreira[1] and Rui J. B. Bessa[2,3]

[1]*Instituto Nacional de Investigação Agrária e Veterinária, Pólo Investigação da Fonte Boa (INIAV-Fonte Boa), 2005-048 Santarém, Portugal.* [2]*Centro Investigação Interdisciplinar em Sanidade Animal (CIISA), Avda Universidade Técnica, 1300-477 Lisboa, Portugal.* [3]*Faculdade de Medicina Veterinária, Universidade Lisboa (ULisboa), Avda Universidade Técnica, 1300-477 Lisboa, Portugal.*

Abstract

Aim of study: To evaluate the impact of *Cistus ladanifer* L. (rockrose) tannins on ruminal degradability and fermentation characteristics and the use of polyethylene glycol (PEG), as feed additive, to mitigate the anti-nutritional effects of rockrose tannins.

Material and methods: Aerial parts of rockrose plants were harvested in March, freeze dried and divided in 4 subsamples which were treated with 0, 25, 50 and 75 g of PEG/kg of dry matter (DM). The mixtures were analysed for chemical composition including total phenolics, total tannins and condensed tannins. *In situ* rumen organic matter (OM) and N rumen degradability was evaluated using three rumen-cannulated rams and ruminal fermentation pattern (volatile fatty acids (VFA), gas production) was evaluated *in vitro* using a Rumen Simulation Technique (RUSITEC) apparatus.

Main results: In situ experiment indicated that the effective degradability of the OM and N increased linearly ($p<0.05$) with PEG inclusion due to an increase of the degradation rate ($p<0.05$). RUSITEC data indicated that substrate disappearance and gas and VFA production increased linearly ($p<0.05$) with PEG inclusion.

Research highlights: Inclusion of PEG to *C. ladanifer* feed was effective to prevent the anti-nutritive effects of tannins. Thus, the use of PEG as feed additive can promote a better utilization of this shrub by ruminants.

Additional key words: condensed tannins; rumen degradability; rumen fermentation, PEG, RUSITEC.

Abbreviations used: ADF (acid detergent fiber); ADL (acid detergent lignin); CP (crude protein); CT (condensed tannins); CTp (condensed tannins measured using butanol-HCl method); CTv (condensed tannins measured using the vanillin assay); D (disappearance of nutrients feed from the bag in the *in vitro* study); DM (dry matter); ED (effective degradability); N (nitrogen); NDF (neutral detergent fiber); OM (organic matter); PEG (polyethylene glycol); RUSITEC (Rumen Simulation Technique); TTrd (total tannins measured using the radial diffusion method); VFA (volatile fatty acids).

Authors´ contributions: MTPD and RJBB participated in data analysis and interpretations. MTPD designed and conducted the *in situ* experiments. OCM conducted the RUSITEC experiment. All authors prepared and approved the final manuscript.

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Correspondence should be addressed to Maria Teresa P. Dentinho: teresa.dentinho@iniav.pt

Introduction

In the south of Portugal, during the summer and fall/early winter, pastures are almost non-existent or with low productivity and poor nutritional value. The dominant vegetation during those periods is trees and shrubs, evergreen plants less sensitive to variations in temperature and rainfall. This type of vegetation has always been an important source of primary nutrients for grazing animals. However, in relation to other feed sources, shrubs have lower nutritive value because they usually contain high levels of parietal compounds and anti-nutritional compounds, particularly tannins. Despite their poor nutritional quality, the inclusion of shrubby species in ruminant diets assumes a high importance in the Mediterranean area in order to control the combustible vegetation as fire prevention and to maintain animals reducing feeding costs mainly during the periods when the feed deficits require expensive supplementation.

Cistus ladanifer L., commonly known as rockrose, is a shrub mainly distributed around the Mediterranean basin and very abundant on the Iberian Peninsula including the central and southern regions of Portugal (Simões *et al.*, 2008). In recent decades, due to land abandonment and recurrent fire events, the area

occupied by rockrose has expanded considerably (Robles & Garzino, 2000; Mendes *et al.*, 2015) leading to loss of biodiversity and further increase in forest fire risk (Zarovali *et al.*, 2007; Mendes *et al.*, 2015). The reduction in species diversity in the rockrose area is attributed both to the allelopathic activity of rockrose, in which toxic compounds are produced and released into the environment inhibiting the development of other plants, and to the degree of soil degradation, which limits the installation of tree species (Meireles *et al.*, 2005; Sosa *et al.*, 2010).

Cistus ladanifer is highly combustible, and thus might contribute to the initiation and spread of forest fires. The uncontrolled growth of the rockrose is fought by the farmers by cutting, shredding and removing the vegetal material (Bruno Soares, 2008). These operations are expensive and must be performed regularly, to be efficient. Due to the low profitability of the farms and forests where rockrose develops, the costs of these cleaning operations are difficult to recover (Mendes *et al.*, 2015). Thus, valorisation of *C. ladanifer* as animal feed, either by direct browsing by ruminants or through its incorporation in compound feeds would benefit the sustainability of the wood-pasture systems. Nevertheless, although widely available throughout the whole year, the rockrose is little grazed. Nutritionally, rockrose is an unbalanced feed, with low crude protein (CP) and low organic matter (OM) digestibility and high levels of condensed tannins (CT) (Guerreiro *et al.*, 2016).

Tannins are water soluble polyphenolic polymers that have the ability to complex with numerous types of molecules including proteins, polysaccharides, and minerals (Min & Hart, 2003; Patra & Saxena, 2011; Le Bourvellec & Renarda, 2012). When present in high concentrations in livestock diets, tannins reduces intake and digestibility of proteins and carbohydrates by inhibiting the activity of the microorganisms in the rumen and of the digestive enzymes, reducing the performance of animals (Silanikove *et al.*, 1996; Patra & Saxena, 2011; Yisehak *et al.*, 2014).

Polyethylene glycol (PEG) is a polymer that binds irreversibly to tannins preventing the tannin-protein complexation. PEG has been used to study the tannins anti-nutritional activity and as additive in high tannins feeds to improve the voluntary feed intake, digestibility and animal performance (Priolo *et al.*, 2000, 2002; Ndagurwa & Dube, 2013). The amount of PEG needed to neutralize the effects of feed tannins depends on the concentration and reactivity of tannins. This has not yet been established for *C. ladanifer* tannins.

This study aims: 1) to evaluate the impact of tannins on in vitro rumen fermentation characteristics and on *in vitro* and *in situ* ruminal degradability of rockrose; 2) to determine the quantity of PEG that must be added to rockrose to remove tannins anti-nutritive effects allowing its use in ruminant diets.

Material and methods

The aerial parts of rockrose plants (leaves and soft steams) were harvested in March in the south Portugal coast (37° 57' N, 8° 45' W). Immediately after collection, plant material was transported to the laboratory of INIAV-Fonte Boa, and kept frozen at -20°C and freeze dried. The plant material was ground, and passed through a 3 mm sieve and divided into 4 subsamples which were treated with 0, 25, 50 and 75 g of PEG /kg of dry matter (DM). The average molecular weight of PEG was of 20000 (BDH Laboratory Supplies, Poole, England). The mixtures were analysed for chemical characterization and the results are shown in Table 1.

Three Merinos rams, 4 yr old, with mean live weight of 52.3 (±3.4) kg, fitted with permanent ruminal cannula, were used for *in situ* degradability study and as rumen fluid donors for the *in vitro* study. Animals were offered daily 45 g of DM/ kg of metabolic weight of lucerne pellets containing: 178 g/kg DM of CP; 462 g/kg DM of neutral detergent fibre (NDF); 294 g/kg DM of acid detergent fiber (ADF) and 132 g/kg DM of ash. Rams were fed twice daily (09.30 and 17.30 h) in two equal portions. Clean water and vitamin-mineral blocks were always available.

In vitro RUSITEC study

Rumen fluid for *in vitro* study was collected from the animals immediately before the morning feeding, mixed and strained through four layers of cheesecloth, into a pre-heated flask with an O_2-free headspace, to separate the liquid and solid fractions.

Samples of rockrose untreated and treated with PEG were incubated *in vitro* using a semi-continuous fermentation system, adapted from the Rumen Simulation Technique (RUSITEC), conceived by Czerkawski & Breckenridge (1977). The *in vitro* system was composed of four vessels with an effective volume of 1 L. On the first day, each one was inoculated with 500 mL of strained rumen juice, 200 mL of artificial saliva (McDougall, 1948) and 100 mL of deionized water, under CO_2 flux. To start the incubation, one nylon bag (14 × 7 cm, 150 µm pore size) containing squeezed solid rumen contents (80 g weight) and other similar nylon bag containing the substrate (15 g) were placed in each vessel. After 24 h the bag with the solid inoculum was withdrawn

and replaced by a new one containing the substrate. Subsequently, each bag was replaced by a new one after 48 h incubation periods. The removed bags were squeezed and washed with 80 mL artificial saliva and the washing returned to the vessel. The liquid effluents and fermentation gases left the vessels by overflow and were recovered in 2 L capacity flasks, placed in a water bath at 4ºC. There, gases were separated from the liquid effluents and were recovered in graduated glass columns filled with water. The vessels received a continuous infusion of McDougals' buffer solution (pH 7.0), complemented with 0.57 g/L of ammonia sulphate, at a rate of 42 mL/h. The RUSITEC was operated for 5 days to allow the adaptation of the microorganisms to the feed and to the conditions of the system, and measurements were done in the following 8 days. Daily, gas production and volume of effluents were registered and the bags removed from the RUSITEC were washed with deionized water and dried to constant weight in an air circulation oven, at 60ºC. After measuring the total volume of the liquid effluents, sub samples were collected daily, for analysis.

Ruminal *in situ* degradability

Samples of rockrose untreated and treated with PEG were weighed (3 g) in duplicate into nylon bags (80 mm × 140 mm; Estal Mono 127 T/154 (305/61") with pore sizes of 40 µm diameter. The bags were incubated in duplicate in the rumen of the cannulated rams before the morning feed for 2, 4, 6, 8, 16, 24, 48, 72 and 96 h. After incubation, the bags were washed in cold tap water and dried to constant weight at 45ºC in a forced air oven. Zero-time losses were estimated by washing three bags per sample without previous ruminal incubation. The disappearance values of OM and CP were fitted to the Ørskov & McDonald´s (1979) exponential model $D = a + b\,(1\text{-}\exp^{-ct})$, where D is the percentage of OM or CP that disappear from the bag at time a given (t), and a represents the soluble or rapidly degradable fraction of the sample, b represents the non-soluble degradable fraction which disappears at a constant fractional rate c per unit of time. The effective degradability (ED) was calculated according to the following equation ED = a + [bc/(c + k)] where k is the outflow rate from the rumen and was assumed to be either 2 or 5% h^{-1}, which is representative for low and medium level of feeding (1× maintenance and <2 times the maintenance requirements) according to AFRC (1993).

Chemical analysis

Samples of rockrose untreated and treated with PEG were analysed in duplicate for determination of DM (ISO 6496, 1999), ash (ISO 5984, 2002), total nitrogen (N) (ISO, 5983, 1997), sugar and starch (Clegg, 1956). NDF, ADF and acid detergent lignin (ADL) were determined according to Goering & Van Soest (1970). NDF was assayed with sodium sulphite, without alpha amylase and expressed with residual ash. The minerals Ca, Na, K and Mg were analysed by atomic absorption spectrometry (ISO 6869, 2000) and P by UV/vis spectrometry (ISO 6491, 1998).

The extraction of phenolic compounds was carried out in four replicates as described by Khazaal *et al.* (1993). The extract obtained was used for total phenols, total tannins, and condensed tannins assays. Total phenols and tannins 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. Condensed tannins were measured using the vanillin assay (CTv) of Broadhurst & Jones (1978) and using the proanthocyanidin assay (butanol-HCl method) (CTp) (Porter *et al.*, 1986). Condensed tannins of vanillin method are expressed as catechin equivalent, using catechin (Sigma C-1788) as standard and CTp as absorbance read at 550 nm. Also total tannins were measured by a protein precipitation assay, the radial diffusion method (TTrd) performed in agarose plates with a protein, the bovine serum albumin (Sigma A-7906) using tannic acid as standard (Hagerman, 1987).

Bag residues of the *in situ* and *in vitro* studies were analysed for DM, ash, and N; and bag residues of *in vitro* study were also analysed for NDF according the aforementioned methods. The effluents from fermenters were analysed for volatile fatty acids (VFA) concentrations were determined in 1.25 mL strained rumen fluid after addition of 0.25 mL ortophosphoric acid solution (25%) and centrifugation at 15,000 g for 10 min at 4 ºC. The supernatant was analysed by gas chromatography using a gas chromatograph HP6890 series (Hewlett-Packard, Avondale, PA, USA) equipped with a flame ionization detector and a semi-capillary column (MN 116; Permabond-FFAP, Macherey-Nagel GmbH & Co. KG, Düren, Germany) with 50 m, 0.25 mm internal diameter and 0.25 µm film thickness. The chromatographic conditions were as follows: injector temperature, 230 ºC; detector temperature, 220 ºC; helium was used as carrier gas at constant flow of 1.0 mL/min and the split ratio was 1:50. The oven temperature program was: 45 ºC (maintained for 2 min), followed by a 10 ºC/min ramp to 220 ºC (maintained for 20 min). Volatile fatty acids were identified by comparison with retention times of known standards (Sigma-Aldrich Inc., St. Louis, MO, USA) and quantified by external standard calibration.

Table 1. Chemical composition (g/kg DM) of rockrose and rockrose mixed with 0, 25, 50 and 75 g/kg dry matter (DM) of polyethylene glycol (PEG).

	PEG addition (g/kg DM)			
	0	**25**	**50**	**75**
DM (g/kg)	905	909	916	917
Organic matter	959	960	961	962
Crude protein	70.4	68.8	66.5	64.9
Sugar	57.4	61.5	61.8	59.6
Starch	47.8	52.3	47.3	45.4
NDF[1]	301	308	376	384
ADF[1]	272	242	249	248
ADL[2]	83.9	76.9	87.3	87.7
Minerals				
Ca	5.80	5.61	5.62	5.07
P	1.32	1.26	1.26	1.20
Na	0.59	0.48	0.60	0.62
K	4.53	4.90	4.59	3.87
Mg	1.93	1.87	1.75	1.64
Total phenols[3]	108	87.3	67.4	67.4
Total tannins[3]	87.7	70.2	48.4	47.2
Condensed tannins (vanillin)[4]	69.1	28.9	nd[6]	nd
Condensed tannins (proanthocyanidins)[5]	207	138	82.6	80.5
Total tannins (radial diffusion)[3]	43.8	43.3	nd	nd

[1] NDF, ADF: neutral detergent and acid detergent fiber, respectively. [2] ADL: acid detergent lignin. [3] tannic acid equivalent in g/kg DM. [4] catechin equivalent in g/kg DM. [5] abs_{550nm} /g DM. [6] nd: not detected.

Statistical analysis

The effect of PEG inclusion on the *in vitro* DM, OM, N, and NDF disappearance and ruminal fermentation characteristics (VFA, and gas production) was analysed according to the following model:

$$Y_{ij} = \mu + P_i + e_{ij,}$$

where Y_{ij} = dependent variable, μ = overall mean, P_i = effect of PEG inclusion (i= 0, 25, 50, 75g/kg of PEG in DM), and e_{ij} = residual error. The linear and quadratic effects of PEG inclusion were evaluated through polynomial orthogonal contrasts.

Ruminal degradation parameters (*a*, *b*, *c*) and effective degradability values were obtained after fitting the exponential model to the *in situ* data using the GraphPad Prism software (Motulsky & Christopoulos, 2003) of each sample type in each ram, and thereafter analysed using the Proc mixed procedure (SAS Inst., 2004) using the follow statistical model:

$$Y_{ijl} = \mu + A_i + P_j + \varepsilon_{ijl,}$$

where Y_{ij} is the dependent variable; μ the overall mean; A_i the animal as random block (i = 1, 2, 3); P_j the fixed effect of PEG inclusion (j = 0, 25, 50, 75 g/kg of PEG in DM) and ε_{ij} the random error. The linear and quadratic effects of PEG inclusion were evaluated through polynomial orthogonal contrasts.

Results

The chemical composition of rockrose and rockrose treated with PEG is presented in Table 1. The concentration of total phenols, total tannins and condensed tannins decreased and the concentration of NDF increased with the increasing inclusion of PEG. For the higher levels of PEG (50 and 75 g/kg DM), the condensed tannins were undetectable either by vanillin and radial diffusion methods.

The effects of PEG inclusion on nutrient disappearance from the bags in RUSITEC and the production of gas and VFA are shown in Table 2. The disappearance of DM and OM increased linearly (p<0.001) with the level of PEG inclusion. The disappearance of NDF presented

Table 2. Effect of addition of PEG to rockrose on disappearance (%) of feed material after 48 h of incubation on daily production of volatile fatty acids (VFA, mmol/d) and gas (L/d) on the RUSITEC.

	PEG addition (g/kg DM)				SEM[1]	Contrasts[2]	
	0	25	50	75		Linear	Quad.
Disappearance							
Dry matter	35.1	38.9	39.0	41.4	0.63	< 0.001	0.253
Organic matter	36.7	40.0	39.9	42.1	0.63	< 0.001	0.399
Nitrogen	12.3	12.8	12.7	14.7	1.20	0.189	0.546
NDF[3]	-37.6	-30.3	-5.40	-0.96	1.194	< 0.001	0.241
VFA							
Acetate	8.57	16.6	19.3	22.4	2.138	< 0.001	0.256
Propionate	3.01	6.24	6.07	6.84	0.576	< 0.001	0.041
Butyrate	0.33	0.94	1.30	1.23	0.136	< 0.001	0.020
Valerate	0.18	0.27	0.28	0.31	0.070	0.218	0.686
Caproic	0.14	0.06	0.05	0.06	0.040	0.137	0.294
Total	12.2	24.2	27.0	30.9	2.738	< 0.001	0.152
A:P ratio[4]	2.84	2.66	3.14	3.25	0.135	0.008	0.305
Gas	1.63	1.87	1.94	2.02	0.056	< 0.001	0.198

[1] SEM: standard error of the means. [2] linear and quadratic orthogonal polynomial contrasts.
[3] NDF: neutral detergent fiber. [4] acetate: propionate ratio.

negative values for all levels of PEG inclusion but nevertheless increased linearly (p<0.001) with the level of PEG. Also the production of acetate, and total VFA, as well as the gas production increased linearly (p<0.001) with PEG inclusion. The acetate/propionate ratio also increased linearly (p = 0.008) with the level of PEG inclusion. PEG treatment increased the production of propionate and butyrate according to a quadratic pattern. The production of valerate and caproic acids were not affected by PEG.

The *in situ* OM and N rumen degradation kinetic parameters and the effective degradability (ED), computed assuming a ruminal outflow rate of 2%/h (ED2) and 5%/h (ED5), observed for the rockrose and rockrose-PEG mixtures are presented in Table 3. The addition of PEG to rockrose, resulted in a linear increase (p<0.03) of the fractional rate of degradation (c) and of ED2 and ED5 of OM and CP. The soluble or rapidly degradable fraction "a" was not affected linearly by PEG inclusion, although a quadratic effect was observable for OM. The fraction potentially degradable in the rumen (b) decreased linearly (p<0.05) with PEG addition.

Discussion

The CP concentration of the aerial part of rockrose was close to the recommendation of the NRC (1985) for maintenance sheep diets (70 *vs* 80 g/kg of DM). Moreover, fibre concentration was moderate and much less than most of grass hays used in ruminant nutrition (*i.e.* 300 g NDF/kg DM *vs* 500 to 600 g NDF/kg in many hays). Despite that, the *in vitro* OM digestibility of rockrose has been reported to be as low as 30 % (Guerreiro *et al.*, 2016). Our data on OM disappearance after *in vitro* incubation with rumen contents for 48 h was also fairly low (35%), although the *in situ* data suggest much higher rumen degradation. One of the reasons for low digestibility of rockrose could be attributed to the presence of tannins in high concentration. In fact, rockrose samples presented concentrations of 108 g/kg DM of total phenols and of 69 g/kg DM of condensed tannins. Concentrations of phenolics above 80 g/kg DM and of condensed tannins above 50 g/kg DM are likely to be detrimental for animal nutrition, depressing feed digestibility and overall nutrient availability for the animals (Rubanza *et al.*, 2005; Patra & Saxena, 2011). One approach to test the hypothesis that CT are the main responsible for the low nutritive value of rockrose is to neutralize their anti-nutritive effects, using PEG as binding substrate. PEG is a non-nutritive polymer that has the ability to irreversibly bind tannins and thus decrease their ability to interact with dietary nutrients and microorganisms, increasing the ruminal degradation and fermentation of tannins rich feeds (Theodoridou *et al.*, 2010). PEG also has the capacity to release protein from the already formed tannin-protein complexes (Barry & Manley, 1986). In fact, due to the formation of PEG-tannin complexes major changes in the chemical composition of rockrose samples were observed, such as the reduction of detectable

Table 3. Effect of addition of PEG to rockrose on *in situ* degradation kinetic parameters and effective degradability (ED) of organic matter and crude protein.

	PEG addition (g/kg DM)				SEM[1]	Contrasts[2]	
	0	25	50	75		Linear	Quad.
Organic matter							
a[3]	36.9	36.4	35.9	37.8	0.35	0.190	0.014
b[4]	45.6	44.0	44.7	43.1	0.42	0.022	0.917
c[5]	2.3	3.5	4.8	4.9	0.20	0.001	0.208
ED2[6]	61.3	64.4	67.3	68.3	0.31	< 0.001	0.204
ED5[7]	51.3	54.6	57.7	59.0	0.44	< 0.001	0.357
Crude protein							
a[3]	16.5	15.0	19.1	16.0	1.78	0.842	0.787
b[4]	70.7	68.4	58.2	60.2	2.77	0.048	0.612
c[5]	1.3	1.8	2.7	3.5	0.41	0.003	0.694
ED2[6]	44.5	47.3	52.4	53.5	0.99	0.003	0.651
ED5[6]	31.2	33.1	39.4	40.2	1.09	0.010	0.826

[1] SEM: standard error of the means. [2] linear and quadratic orthogonal polynomial contrasts. [3]*a*, the rapidly degraded fraction, expressed as % of OM or CP present at initial time. [4]*b*, slowly degraded fraction, expressed as % of OM or CP present at initial time. [5]*c*, rate of degradation of the *b* fraction, % per h. [6]ED2, ED5: Effective degradability computed using a rumen fractional outflow rate of 2% and 5% per h, respectively.

total phenols, total and condensed tannins and also the increase of NDF. Interactions with the insoluble matrix, proteins, polysaccharides and other polymers can decrease the solubility of tannins in the extractant (acetone/water in our study), underestimating tannin content of feeds (Scalbert, 1992; Cerpa-Calderon & Kennedy, 2008; Hanlin *et al.*, 2010). Also the higher NDF in rockrose treated with PEG should result from the formation of PEG-tannin complexes which are insoluble in the neutral detergent solution appearing in the NDF fraction leading to their overestimation (Makkar *et al.*, 1995).

The increased addition of PEG to rockrose samples led consistently to an increase of the *in situ* factional rate of degradation and ED as well as the OM disappearance, VFA and gas production in RUSITEC, which is a clear indication that condensed tannins were a limiting factor on ruminal digestion of rockrose.

The effect of tannins on protein degradation is usually associated to a reduction in the soluble fraction or immediately degradable fraction *a* and to a reduction of the fractional rate of degradation *c* (Frutos *et al.*, 2004). In our study, the increase of degradability of N resulted from a marked increase in the rate of degradation *c*, and not by a decrease of the *a* fraction. The effects of tannins on nutrient degradability depend not only of their complexation properties but also on their effect on the microbial population and on its enzymatic activity (McSweeney *et al.*, 2001). The reduction of feed rumen degradation rates induced by tannins have been associated either with the reduction of the attachment of microbes to feed particles or to a specific inhibition of microbial growth and enzyme activity (Makkar *et al.*, 1988; McAllister *et al.*, 1994; McSweeney *et al.*, 2001).

As mentioned above, the Van Soest detergent system of fibre analysis is not adequate for tannin-rich feeds because the non-removal of tannin-protein complexes by the detergents leads to misleading values of fibre (Makkar *et al.*, 1995). This explains the negative disappearances of NDF found in the RUSITEC experiment. Nevertheless, the linear increase of *in vitro* NDF disappearance at 48 h and of the acetate acetate: propionate ratio with increasing PEG inclusion suggest that tannins also have negative effects on fibre digestion. In fact, several studies have shown that fibre degradation and acetate production in the rumen can be drastically reduced in animals that consume tannin rich feeds due to substrate privation, enzymatic inhibition or by direct action on rumen microorganisms (Frutos *et al.*, 2004; Pellikaan *et al.*, 2011; Castro-Montoya *et al.*, 2011).

The results obtained suggest that the use of PEG as feed additive can mitigate the anti-nutritional effects of tannins being an approach to value this resource and thus contributing to minimize the risk of forest fires, to reduce the animal feed costs and to improve the local economy. *In vitro* and *in situ* studies are simplified experimental models that do not capture the complexity of the animal and of animal×pasture interactions. Thus more studies should be conducted to evaluate the usefulness of PEG supplements in practical production conditions.

The results obtained confirm that the high content of tannins in rockrose, is a major limiting factor of its ruminal digestive utilization. The addition of PEG to ground rockrose seems to be a good approach to neutralize the tannins present, allowing for a better utilization of this shrub by ruminants. The inclusion of 50 g/kg DM of PEG in rockrose appears to be sufficient to prevent negative effects of rockrose tannins, although the improvement followed a linear relationship with PEG inclusion. The results suggest that rockrose can be used as animal feed which may be a way to control this shrub contributing to a lower accumulation of biomass with high combustibility, thus reducing the likelihood and the impacts of fires.

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