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in

Casasús I. (ed.), Lombardi G. (ed.).
Mountain pastures and livestock farming facing uncertainty: environmental, technical and socio-economic challenges

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 116

2016
pages 291-294

Effect of forage conservation and inclusion of condensed tannins on *in vitro* gas and methane production

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Abstract. Condensed tannins can reduce methane emissions coming from the ruminal fermentation of forage. The aim of the study was to evaluate the effect of forage conservation (hay *vs.* fresh) and the inclusion of condensed tannins [CT, 10% of Quebracho (*Schinopsis Balansae*) with 75% of CT] in the concentrate (Control *vs.* CT) on *in vitro* gas and methane production and the ruminal degradability of organic matter (IVOMD) at 24 h. The diets contained 70% forage and 30% concentrate. Gas production (ml/g organic matter (OM)) was not affected by the forage conservation or tannin inclusion in the concentrate (P>0.05). Regarding the kinetic parameters of gas production, the asymptotic gas production was similar in both forages, but the gas production rate was greater in pasture than in hay (P<0.001). The kinetic parameters were not affected by tannin inclusion. Methane production (ml/g OM) was affected by the interaction between the forage conservation and concentrate (P<0.01). Methane production decreased by 20.5% when tannin was included in the hay diet but did not change in the pasture diet (P>0.05). The IVOMD was only affected by the forage conservation in the diet, being greater for the diet with fresh *vs.* hay pasture (74.1% and 61.6%, respectively; P<0.001). In conclusion, the use of concentrate with condensed tannins in ewes' diets reduced methane production in diets containing hay but not in those based on pasture. Gas production and IVOMD were unaffected by the inclusion of condensed tannins in the diet, regardless of the conservation of forage

Keywords. *In vitro* digestibility – Fermentation kinetics – Ovine – Quebracho – Condensed tannins.

Effet de la conservation du fourrage (foin* vs. *frais) et de l'inclusion des tanins condensés dans la production* in vitro *de gaz et méthane

Résumé. *Les tanins condensés peuvent réduire les émissions de méthane provenant de la fermentation ruminale de fourrage. L´objectif de l'étude était d'évaluer l'effet de la conservation du fourrage (foin* vs. *frais) et de l'inclusion des tanins condensés [TC, 10% de Quebracho* (Schinopsis balansae) *avec 75% de tanins condensés] dans le concentré (par rapport au témoin QUE) sur la production* in vitro *de gaz et méthane et la dégradabilité ruminale de la matière organique (DRMO) à 24 h. Les régimes contenaient 70% de fourrage et 30% de concentré. La production de gaz (ml/g de MO) n'a été pas affectée par la conservation du fourrage ou par l'inclusion des TC dans le concentré (P>0,05). En ce qui concerne les paramètres cinétiques de production de gaz, la production de gaz asymptotique était similaire dans les deux fourrages, mais le taux de production de gaz était supérieur pour le fourrage frais par rapport au foin (P<0,001). Les paramètres cinétiques n'étaient pas affectés par l'inclusion des TC. La production de méthane (ml/g OM) a été affectée par l'interaction entre la conservation du fourrage et l'inclusion des TC (P <0,01). La production de méthane a diminué de 20,5% avec l'inclusion des TC dans le concentré dans le régime de foin, mais n'a pas changé dans le régime de fourrage frais (P> 0,05). La DRMO a été affectée par la méthode de conservation du fourrage, étant supérieure pour le régime de fourrage frais* vs. *foin (74,1% et 61,6%, respectivement; P<0,001). En conclusion, l'inclusion de concentré avec des tanins condensés dans l'alimentation des brebis réduit la production de méthane dans les régimes constitués par du foin, mais pas dans ceux qui sont constitués par du pâturage frais. La production de gaz et l'IVOMD n'ont pas été affectées par la présence de tanins condensés dans l'alimentation, quelle que soit la méthode de conservation du fourrage.*

Mots-clés. *Digestibilité in vitro – Cinétique de fermentation – Ovin – Quebracho – Tanins condensés.*

I – Introduction

Ruminants are responsible for 28% of methane (CH_4) emissions, which are related to global warming and climate change (IPCC, 2013). In ruminant, the production of methane during enteric fermentation contributes to an important loss of energy of up to 12% (Johnson and Johnson, 1995). Thus, strategies to reduce the CH_4 production during the enteric fermentation and to improve feed efficiency have been studied. Condensed tannins (CT) are a diverse group of polymeric flavanols with functional groups that chelate metal ions and are capable of binding protein and fibre components of feed as well as interacting with microbial populations of the rumen (Min *et al*., 2003). Their presence in the diet can reduce enteric methane emissions, as it has been observed *in vitro* to different extents (Hassanat and Benchaar, 2013). However, tannins have a wide variety of chemical structures, which may explain their different effects on methano-genesis, which is also depending on the dose, the used substrate and the type of tannin (Getachew *et al*., 2008). The objective of this experiment was to study the effects of inclusion of CT in the concentrate in diets using forages with different conservation on rumen fermentation characteristics.

II – Materials and methods

Two forages (fresh *vs.* hay pasture) and two concentrates (control *vs*. CT) were used to create four diets, each consisting of 70% forage and 30% concentrate. Fresh and hay pasture were obtained in La Garcipollera Research Station, in the mountain area of the southern Pyrenees (North-Eastern Spain, 42º 37´ N, 0º 30´ W, 945 m a.s.l.). The pasture was composed of 68% grass, 20% legumes and 12% of other species. The meadow was harvested in October 2013 to produce hay (75 g CP/kg DM and 680.5 g NDF/kg DM) whereas it was sampled in April 15, 2014 to obtain fresh pasture (263.2 g crude protein (CP)/kg dry matter (DM) and 487.6 g neutral detergent fibre (NDF)/kg DM) samples. The Control (C) concentrate was a commercial concentrate for ewes (153.3 g CP/kg DM and 1.03 eq-g cyanidin/kg DM). The CT concentrate (155.1 g CP/kg DM and 7.67 eq-g cyanidin/ kg DM) was similar to C but contained 10% of Quebracho extract (SYLVAFEED ByPro Q, Spain) with 75% of condensed tannins. All ingredients were dried at 60 °C and ground through a 1-mm sieve (ZM200 Retsch, Germany). The resulting diets were: hay + C; hay + CT, Fresh + C and Fresh + CT.

In vitro gas production was carried out using rumen fluid collected from 4 rumen fistulated rams fed 70% alfalfa hay and 30% barley grain. The rumen fluid was strained through 4 layers of cheesecloth and mixed with the buffer solution, based on the protocol of Menke and Steingass (1988). Three 0.5 g DM sub-samples of each diet were incubated in bottles with 60 ml of incubation solution in a water bath (39 ºC) for 24 h. After this period, 8 ml of gas were collected in 5 ml tubes to quantify methane production. At the end of gas sampling, the fermentation was stopped. To study the fermentation kinetics, gas production was recorded at 1 h intervals during 24 hours (Ankom Technology, NY, USA). The data for the nonlinear gas production were fitted to the following exponential equation (Orskov and Mcdonald, 1979): $P = A*(1-e^{-ct})$, where P is the cumulative gas production (ml) at time t (h), A is the potential gas production (ml g-1) and c is the rate of gas production (ml h-1). Methane was analysed with gas chromatograph HP-4890, equipped with a capillary column TG-BOND Q+ (Thermo Scientific). The temperature of the inlet, the detector and the oven was maintained at 200, 250 and 100 ºC, respectively. Methane identification was based on the retention time as compared with the standard.

In vitro organic matter digestibility (IVOMD) was estimated by filtering residues using pre-weighed bag (50 µm; Ankom, NY, USA). The bags with sample were dried at 103 ºC for 48 h to obtain the dry matter content. After 48 h, bag content was weighed and was placed at 550 ºC for to obtain the ashes. The organic matter of bag content was obtained as DM-ashes and the IVOMD was obtained as: (Incubated OM-bag content OM)/Incubated OM.

Data were analysed with a general lineal model with the forage conservation, the inclusion of CT and its interaction as fixed effects. Differences were significant for P<0.05 and tendencies for P<0.10.

III – Results and discussion

The conservation of forage tended to affect gas production (P=0.06), influenced the rate of gas production (c) (P<0.001) and the IVOMD but did not affect the asymptotic gas production (A) (Table 1). Pasture-based diets had higher rate of gas production and IVOMD than hay-based diets. This can be explained by differences in the quality between fresh and hay pasture, due to differences in the maturity stage and the process of haymaking. The kinetics of gas production and IVOMD depend on the relative proportions of soluble and degradable particles of the feed (Getachew *et al*., 1998).

Table 1. Effect of the inclusion of the concentrate with condensed tannins (CT) or the Control concentrate in a diet with fresh pasture or hay on *in vitro* fermentation characteristics at 24 h

Variables	Forage		Concentrate			P values†	
	Hay	Fresh	Control	CT	s.e.††	Forage	Concentrate
Gas production (ml/g OM)	147.49	161.74	159.82	149.42	14.07	0.06	0.16
Ruminal fermentation kinetics							
A (ml/g)	73.47	66.77	72.93	67.31	8.80	0.15	0.22
c (ml/h)	0.07	0.11	0.09	0.09	0.02	0.001	0.63
IVOMD (%)	61.57	74.07	67.55	68.08	3.96	0.001	0.79

† The interaction between the forage and the inclusion of CT was not significant (P>0.05).
†† Standard error of mean.

The inclusion of CT had no effect on total gas production and IVOMD (P>0.05). Similarly, Hassanat and Benchaar (2013) reported that the inclusion of Quebracho at 20 g/kg in diet did not decrease gas production and IVOMD. The fermentation kinetics showed that the asymptotic gas production (A) and the rate of gas production (c) were not affected by the inclusion of CT in the concentrate as well (Table 1). In contrast, Hervás *et al*. (2000) observed that the inclusion of tannic acid (10%) in soya bean meals reduced both rate and extent of cumulative gas production.

Methane production was affected by the interaction between the forage conservation and inclusion of CT in the concentrate (P<0.05). The inclusion of CT in the diet decreased methane production in the hay–based diet (P<0.05) but did not affect it in the fresh forage-based diet (P>0.05). In this sense, the addition of 20 g Quebracho tannin extract/kg of dietary DM to barley silage failed to reduce enteric methane emissions (Beauchemin *et al*., 2007).

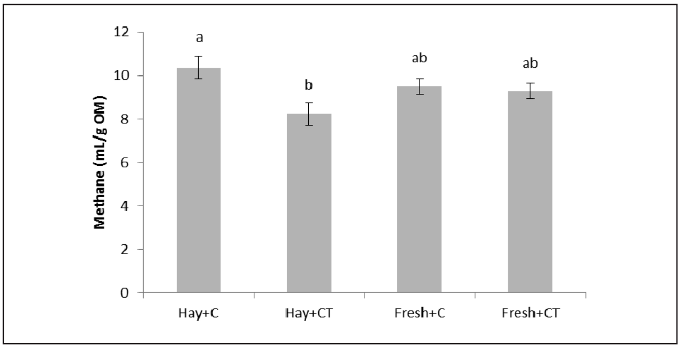

Fig. 1. Effect of the inclusion of the concentrate with condensed tannins (CT) or the Control concentrate in a diet with fresh pasture or hay on methane production after 24 h incubation.

Different superscripts (a,b) denote differences at P<0.05.

A reduction in methane production could be due to a decrease in organic matter degradability (Tan *et al*., 2011). In the present study, IVOMD was not affected by inclusion of CT in the concentrate regardless of forage, thus the reduction of methane production in hay diet can be related to the affinity of CT for feed protein *vs*. microbial protein (Beauchemin *et al*., 2007). The affinity of CT for protozoa and microbial methanogenic population is particularly important due to their role on methane production (McAllister *et al*., 1996). In the current experiment, hay had lower crude protein content than pasture; whereby in hay-based diet the CT could be bound to the methanogenic microbial population instead to link to the feed protein, reducing methane production.

IV – Conclusions

The inclusion of condensed tannins at 22.5 g CT/kg in hay diets could be an interesting alternative in hay-based diets as it reduced *in vitro* methane production with no effect on gas production or IVOMD. However, this inclusion in a fresh pasture diet seems not to be as interesting because it did not reduce *in vitro* production of gas and methane.

Acknowledgments

The authors gratefully acknowledge the staff of the CITA Research Centre for technical support. Research and M. Blanco contract funded by Spanish Ministry of Economy and Competitiveness, INIA (project RTA2012-0080-C00-00) and the European Regional Development Fund. S. Lobón and P.J. Rufino enjoy predoctoral the Government of Aragon and INIA, respectively.

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