Effects of the inclusion of oak tannins in a diet rich in linoleic acid on in vitro rumen biohydrogenation and fermentation in sheep

Carreño D., Hervás G., Toral P.G., Belenguer A., Frutos P.

in

Napoléone M. (ed.), Ben Salem H. (ed.), Boutonnet J.P. (ed.), López-Francos A. (ed.), Gabiña D. (ed.).
The value chains of Mediterranean sheep and goat products. Organisation of the industry, marketing strategies, feeding and production systems

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 115

2016
pages 597-601

Effects of the inclusion of oak tannins in a diet rich in linoleic acid on *in vitro* rumen biohydrogenation and fermentation in sheep

D. Carreño, G. Hervás, P.G. Toral, A. Belenguer and P. Frutos*

Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, 24346 Grulleros, León (Spain)
*e-mail: p.frutos@csic.es

Abstract. There is a lack void of knowledge about which of the many types of tannins might be more specific and useful, in practice, to interfere with rumen biohydrogenation (BH) and modulate the fatty acid (FA) profile of ruminant derived products without impairing diet utilization. Two *in vitro* assays with batch cultures of rumen microorganisms were conducted to analyze the effect of an oenological commercial extract of oak tannins, at a practical dose under farm conditions (2% DM), on the rumen BH and fermentation of a diet also supplemented with 2% DM of sunflower oil. In the first experiment (12 h incubations), freeze-dried ruminal digesta were analyzed for FA composition. In the second one (24 h incubations), rumen fermentation parameters and bacterial community were examined. The addition of 2% DM of the oak tannin extract proved (P<0.01) to be able to slightly reduce the concentration of 18:0 (-5.2%) and increase those of 18:2*n*-6, *n*-3 polyunsaturated FA (PUFA) and *n*-6 PUFA (by approx. +64%), while tended (P<0.10) to decrease *trans*-10 18:1 (-9%) and enhance *trans*-11 18:1 (+14%). These changes were accompanied by increases in the mean value of odd- and branched-chain FA, as well as in some keto-FA concentrations (P<0.05). On the other hand, it had no significant effects on the rumen fermentation characteristics that were analyzed (e.g., gas production kinetic, extent of OM degradation, pH, or ammonia and VFA concentrations; P>0.10). Although positive, when explored at practical doses in terms of animal feeding, results on the use of tannins to modulate microbial BH are not as promising as initially expected. More research in this field is still necessary.

Keywords. Ewe – Fatty acid – Oak tannins - Rumen microbiota – Ruminal fermentation.

Effets de l'inclusion de tanins de chêne dans un régime riche en acide linoléique sur la biohydrogénation et la fermentation ruminale chez les ovins

Résumé. *Il y a un manque de connaissances sur lesquelles de nombreux types de tanins pourraient être plus spécifiques et utiles, dans la pratique, pour interférer avec la biohydrogénation (BH) ruminale et moduler le profil en acides gras des produits de ruminants sans nuire à l'utilisation du régime. Deux essais* in vitro *avec des cultures non-renouvelées de microorganismes du rumen ont été conduits pour analyser l'effet d'un extrait œnologique commercial de tanins de chêne, à dosage pratique dans des conditions d'exploitation (2% MS), sur la BH et la fermentation ruminale d'un régime supplémenté en huile de tournesol (2% MS). Dans la première expérience (12 h d'incubation), la composition en acides gras (AG) des digesta ruminales lyophilisées a été analysée. Dans la deuxième expérience (24 h d'incubation), la fermentation et la communauté bactérienne ruminale ont été examinées. L'addition d'un 2% MS de tanins de chêne s'est avérée (P<0,01) efficace pour réduire légèrement la concentration de 18:0 (-5,2%) et accroitre celles de 18:2n-6, acides gras polyinsaturés (AGPI) n-3 et AGPI n-6 (d'environ +64%), tandis qu'il y avait une tendance (P<0,10) à diminuer le trans-10 18:1 (-9%) et à augmenter le trans-11 18:1 (+14%). Ces modifications n'ont pas été accompagnées d'incréments de la teneur moyenne en AG impairs et ramifiés, ainsi qu'en certains céto-AG (P<0,05). Toutefois, elle n'a pas eu des effets sur les caractéristiques de la fermentation ruminale analysées (par exemple, paramètres de la cinétique de production de gaz, extension de la dégradation, pH ou concentrations d'ammoniac et AGV; P>0.10). Malgré ces effets positifs, les résultats de l'utilisation de tanins à dose pratique ne sont pas aussi prometteurs comme initialement prévu. Il faudrait encore approfondir les recherches à ce sujet.*

Mots-clés. *Brebis – Acide gras – Tanins de chêne – Microbiote ruminale – Fermentation ruminale.*

I – Introduction

Some *in vitro* studies have suggested that the supplementation of ruminant diet with tannins could modulate ruminal unsaturated fatty acid (FA) metabolism, enhancing the accumulation of *trans*-11 18:1 in the rumen due to an inhibition of the last step of biohydrogenation (BH; Vasta *et al*., 2009; Buccioni *et al*., 2011). This 18:1 isomer will act as a precursor of the potentially health-promoting *cis*-9 *trans*-11 conjugated linoleic acid in the animal's own tissue, which would therefore increase its content in milk or meat (Shingfield *et al*., 2008). Nevertheless, other studies on tannins have reported a general inhibition of the BH process instead of a specific inhibition of the reduction of *trans*-11 18:1 to 18:0 (Kronberg *et al*., 2007; Minieri *et al*., 2014). Given the heterogeneity in the structural features and consequently in the reactivity of these phenolic compounds (Mueller-Harvey, 2006), inconsistent results might be explained by distinct effects of different types of tannins on rumen microbiota.

Thus, this *in vitro* study was conducted to investigate the effect of an oenological commercial extract of oak tannins, added to a diet rich in linoleic acid at a practical dose under farm conditions (2% diet DM) on rumen BH, fermentation and bacterial community in sheep.

II – Materials and methods

Two *in vitro* assays were conducted in batch cultures with rumen fluid collected from 5 ruminally cannulated Merino sheep fed a total mixed ration (TMR; forage:concentrate ratio 50:50). After an adaptation period of 15 days, the inocula (collected in three different days, each one corresponding to a replicate) were obtained before the morning feeding and mixed (1:2) with phosphate-bicarbonate buffer. The incubated substrate (6.5 mg/mL of buffered rumen fluid) was the TMR supplemented with 2% DM of sunflower oil plus 0 (control) or 2% DM of a hydrolysable tannin extract (oak; *Quercus robur* and *Q. petraea* - Robletan FST, Agrovin S.A., Spain).

The first experiment was carried out to study ruminal BH, using 16 mL Hungate tubes that contained 12 mL of buffered rumen fluid. After 12 h of incubation (when, according to previous preliminary assays, effects were better detected), the reaction was stopped by placing the tubes into ice-water. They were then stored at -80ºC until FA analysis. The lipids in freeze-dried *in vitro* ruminal digesta were extracted and converted to FA methyl esters by sequential base-acid catalysed transesterification, and quantified by gas chromatography (Toral *et al.,* 2012).

In the second trial, the effects of the oak tannin extract on rumen fermentation and bacterial community were studied using 125 mL serum flasks (containing 50 mL of buffered rumen fluid). The cumulative gas production (*A*), fractional fermentation rate (*c*) and DM disappearance (DMD) were determined after 72 h of incubation (Frutos *et al*., 2004). Additional flasks were incubated for 24 h to measure fermentation parameters (pH, ammonia concentration and VFA production; Frutos *et al*., 2004) and for microbial DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis using 3 restriction enzymes (*Hha*I, *Msp*I and *Hae*III; Castro-Carrera *et al*., 2014).

Fatty acid composition and fermentation parameters were analysed by one-way ANOVA using the MIXED procedure of SAS (v9.3). Hierarchical clustering analysis based on Jaccard distances was performed (www.r-project.org) to build a dendrogram with relative abundance data derived from T-RFLP. Additionally, a multivariate analysis of variance (MANOVA) of each terminal-restriction fragment (T-RF) was conducted to assess the effect of treatment on the whole bacterial structure.

III – Results and discussion

As shown in Table 1, the addition of the oak tannin extract proved to be able to slightly reduce ($P<0.01$) the concentration of 18:0 (-5.2%) and increase those of 18:2*n*-6 and *n*-6 and *n*-3 PUFA (on average, +64%). In addition, oak tannins tended ($P<0.10$) to enhance *trans*-11 18:1 (+14%)

and decrease *trans*-10 18:1 (-9%), which may have positive implications for human health (Shingfield *et al.*, 2008), but the magnitude of changes was small. Our results are consistent with those of some *in vitro* studies suggesting a general inhibition of BH (Kronberg *et al*., 2007; Minieri *et al.*, 2014), rather than the specific inhibition of the saturation of *trans*-11 18:1 that had been detected in other assays (Vasta *et al.*, 2009, Buccioni *et al.*, 2011).

Table 1. Effect of the dietary inclusion of an oak tannin extract (2% DM) on selected fatty acid (FA) concentration (% total FA) after 12-h *in vitro* incubation with rumen inoculum from sheep

	18:0	*trans*-10 18:1	*trans*-11 18:1	18:2 *n-6*	MUFA[1]	*n-3* PUFA[2]	*n-6* PUFA[2]	OBCFA[3]	13-oxo 18:0
Control	61.2	0.387	5.15	0.877	12.1	0.268	0.981	4.84	0.190
Oak	58.0	0.352	5.86	1.47	12.6	0.431	1.59	5.49	0.294
SED[4]	0.415	0.014	0.312	0.105	0.585	0.026	0.102	0.215	0.028
P-value	0.001	0.064	0.087	0.011	0.513	0.009	0.009	0.040	0.020

[1]Monounsaturated FA; [2]Polyunsaturated FA; [3]Odd- and branched-chain FA; [4]Standard error of the difference.

Significant variations in odd- and branched-chain FA (+13%) may reflect shifts in rumen bacteria, which is further supported by changes in some keto-FA (e.g., 13-oxo 18:0; +55%) compatible with alterations in microbial FA metabolism (Toral *et al.*, 2012). However, the hierarchical clustering analysis did not show a segregation of bacterial profiles based on treatment, as plotted in the dendrogram (Fig. 1), which may be attributed to slight shifts in the abundance of specific bacterial populations that could not be detected with this approach. Although significant variations were not revealed either using MANOVA, the oak treatment induced changes ($P<0.05$) in the relative abundances of a few T-RF, such as increases in some fragments compatible with uncultured species of *Lachnospiraceae*. This family includes species that have been suggested to play a role in microbial BH (Huws *et al.*, 2011; Toral *et al*., 2012).

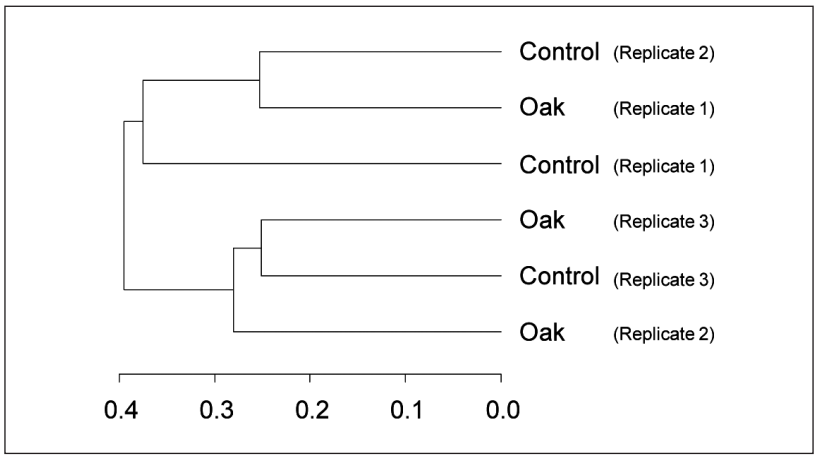

Fig. 1. Effect of the inclusion of an oak tannin extract (2% DM) on the bacterial community: cluster analysis of T-RFLP profiles of total bacteria after 24-h *in vitro* incubation with rumen inoculum from sheep.

The effects of oak tannins on *in vitro* BH of unsaturated FA were not accompanied by any negative effect on rumen fermentation characteristics (P>0.10; see Table 2), which may be explained by the small amount of tannins that was added and their known dose-dependent impact on rumen function (Frutos *et al.,* 2004; Mueller-Harvey, 2006). In this regard, our results support earlier studies demonstrating that the long-lasting generalisation that hydrolysable tannins are more toxic to ruminants and induce less efficient results than condensed tannins would be not only simplistic but also erroneous (see review by Mueller-Harvey, 2006).

Table 2. Effect of the inclusion of an oak tannin extract (2% DM) on rumen fermentation parameters after *in vitro* incubation with rumen inoculum from sheep

	***A* (mL/g OM)**	***c* (/h)**	**DMD (g/g)**	**pH**	**Ammonia (mg/L)**	**Total VFA (mmol/L)**
Control	381	0.068	0.703	6.44	709	79.2
Oak	381	0.071	0.679	6.46	654	71.5
SED†	10.7	0.002	0.030	0.024	101	4.72
P-value	0.979	0.176	0.464	0.526	0.618	0.176

† Standard error of the difference.

IV – Conclusion

The addition of an oak tannin extract, at a practical dose of 2% DM, to a diet rich in linoleic acid modulates *in vitro* microbial BH of unsaturated FA without impairing rumen fermentation. However, these positive effects are not as promising as initially expected.

Acknowledgments

This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO; AGL2011-23700). D. Carreño and P.G. Toral received an FPI predoctoral grant and a *Juan de la Cierva* contract from the MINECO.

References

Buccioni A., Minieri S., Rapaccini S., Antongiovanni M. and Mele M., 2011. Effect of chestnut and quebracho tannins on fatty acid profile in rumen liquid- and solid-associated bacteria: an *in vitro* study. In: *Animal*, 5, p. 1521-1530.

Castro-Carrera T., Toral P.G., Frutos P., McEwan N.R., Hervás G., Abecia L., Pinloche E., Girdwood S.E. and Belenguer A., 2014. Rumen bacterial community evaluated by 454 pyrosequencing and terminal restriction fragment length polymorphism analyses in dairy sheep fed marine algae. In: *J. Dairy Sci*., 97, p. 1661-1669.

Frutos P., Hervás G., Giráldez F.J. and Mantecón A.R., 2004. An *in vitro* study on the ability of polyethylene glycol to inhibit the effect of quebracho tannins and tannic acid on rumen fermentation in sheep, goats, cows, and deer. In: *Aust. J. Agric. Res.*, 55, p. 1125-1132.

Huws S.A., Kim E.J., Lee M.R.F., Scott M.B., Tweed J.K.S., Pinloche E., Wallace R.J. and Scollan N.D., 2011. As yet uncultured bacteria phylogenetically classified as *Prevotella*, *Lachnospiraceae* incertae sedis and unclassified *Bacteroidales*, *Clostridiales* and *Ruminococcaceae* may play a predominant role in ruminal biohydrogenation. In: *Environ. Microbiol.*, 13, p. 1500-1512.

Kronberg S., Scholljegerdes E., Barcelo-Coblijn G. and Murphy E., 2007. Flaxseed treatments to reduce biohydrogenation of alpha-linolenic acid by rumen microbes in cattle. In: *Lipids*, 42, p. 1105-1111.

Minieri S., Buccioni A., Rapaccini S., Pezzati A., Benvenuti D., Serra A. and Mele M., 2014. Effect of quebracho tannin extract on soybean and linseed oil biohydrogenation by solid associated bacteria: an *in vitro* study. In: *Ital. J. Anim. Sci.,* 13, p. 604-608.

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

Shingfield K.J., Chilliard Y., Toivonen V., Kairenius P. and Givens D.I., 2008. *Trans* fatty acids and bioactive lipids in ruminant milk. In: *Adv. Exp. Med. Biol*., 606, p. 3-65.

Toral P.G., Belenguer A., Shingfield K.J., Hervás G., Toivonen V. and Frutos P., 2012. Fatty acid composition and bacterial community changes in the rumen fluid of lactating sheep fed sunflower oil plus incremental levels of marine algae. In: *J. Dairy Sci.*, 95, p. 794-806.

Vasta V., Makkar H.P.S., Mele M. and Priolo A., 2009. Ruminal biohydrogenation as affected by tannins *in vitro*. In: *Br. J. Nutr.*, 102, p. 82-92.