CIHEAM
Options Méditerranéennes

Phytogenic additives to decrease in vitro ruminal methanogenesis

Bodas R., López S., Fernández M., García-González R., Wallace R.J., González J.S.

in

Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). Nutritional and foraging ecology of sheep and goats

Zaragoza : CIHEAM / FAO / NAGREF
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85

2009
pages 279-283

Article available on line / Article disponible en ligne à l'adresse :

---

http://om.ciheam.org/article.php?IDPDF=801018

To cite this article / Pour citer cet article

Bodas R., López S., Fernández M., García-González R., Wallace R.J., González J.S. **Phytogenic additives to decrease in vitro ruminal methanogenesis.** In : Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Morand-Fehr P. (ed.). *Nutritional and foraging ecology of sheep and goats*. Zaragoza : CIHEAM / FAO / NAGREF, 2009. p. 279-283 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 85)

CIHEAM

http://www.ciheam.org/
http://om.ciheam.org/

Phytogenic additives to decrease *in vitro* ruminal methanogenesis

R. Bodas*,, S. López*, M. Fernández*, R. García-González*, R.J. Wallace*** and J.S. González***

*Dpto. Producción Animal, Facultad de Veterinaria, Universidad de León, Campus de Vegazana s/n, 24071 León (Spain)
**ETS Ingenierías Agrarias, Universidad de Valladolid, Avda Madrid 57, 34004 Palencia (Spain)
***Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, Scotland (United Kingdom)

Abstract. Six plants (*Carduus pycnocephalus*, *Populus tremula*, *Prunus avium*, *Quercus robur*, *Rheum nobile* and *Salix caprea*) were tested to determine their effects as feed additives to decrease ruminal methanogenesis *in vitro*. The plants had been selected in a previous screening assay in which a collection of 450 plant species had been tested. Sheep fed alfalfa hay were used as donors for rumen fluid. Batch cultures were performed in 120 ml serum bottles, using 10 ml strained rumen, 40 ml medium, 500 mg DM substrate (50% alfalfa hay, 40% grass hay and 10% barley) and 50 mg DM freeze-dried ground plant additive. After 24 h of incubation, gas production, methane and volatile fatty acids (VFA) production, propionate to acetate ratio, *in vitro* DM digestibility, pH and fermentation efficiency were measured. All the plants tested reduced methane production ($P < 0.001$) in relation to the control (no plant added). *P. avium* and *Q. robur* caused a decrease in propionate production and in propionate to acetate ratio ($P < 0.001$), whereas *S. caprea* reduced propionate production ($P < 0.001$). Gas production, pH and fermentation efficiency were not affected by any of the plants ($P > 0.05$). Among them, *R. nobile* was particularly promising since it showed the greatest and most consistent effect on methane production (–16% in relation to the control, $P < 0.001$). Moreover, it enhanced ruminal fermentation, stimulating DM digestibility ($P < 0.05$) and VFA production ($P < 0.001$) and increasing propionate to acetate ratio ($P < 0.001$). In conclusion, among the plant species evaluated, *Rheum nobile* showed the most consistent effects for decreasing methane production without adversely affecting other parameters of rumen fermentation.

Keywords. Plant additives – Methanogenesis – Rumen fermentation.

***Les additifs végétaux pour réduire la méthanogenèse ruminale* in vitro**

Résumé. *Six plantes (*Carduus pycnocephalus*,* Populus tremula*,* Prunus avium*,* Quercus robur*,* Rheum nobile *et* Salix caprea*) ont été testées afin de déterminer les effets comme additifs pour diminuer la méthanogenèse ruminale* in vitro*. Elles ont été choisies sur la base d'une évaluation de 450 plantes réalisée dans un essai antérieur. Six brebis adultes ont été utilisées comme donneuses de liquide ruminal. Les incubations* in vitro *ont été réalisées dans des bouteilles de sérum de capacité 120 ml. Dans chaque bouteille on a placé 10 ml de liquide ruminal, 40 ml de salive artificielle, 500 mg de matière sèche de substrat (50% luzerne, 40% foin d'herbe et 10% orge) et 50 mg de matière sèche de la plante lyophilisée puis broyée. Après 24 heures d'incubation, la production de gaz, le volume de méthane, les concentrations des acides gras volatils, le rapport propionate/acétate, la digestibilité* in vitro *de la matière sèche, le pH et l'efficacité de la fermentation ont été mesurés. Toutes les plantes étudiées ont réduit la production de méthane ($P < 0,001$).* S. caprea *a réduit la production de propionate ($P < 0,001$). La production de gaz, le pH et l'efficacité de la fermentation n'ont pas été affectés ($P > 0,05$) par toutes les plantes évaluées.* R. nobile *s'est avérée une plante prometteuse, puisqu'elle a entraîné la plus grande chute de la production de méthane (–16% par rapport au témoin, $P < 0,001$). Elle a aussi amélioré la fermentation ruminale, la digestibilité ($P < 0,05$) et la production d'acides gras volatils ($P < 0,001$), et a augmenté le rapport propionate/acétate ($P < 0,001$). Pour conclure, parmi les plantes évaluées,* Rheum nobile *s'est avérée la plante la plus efficace dans la mesure où elle a permis de réduire au maximum la production de gaz sans pour autant affecter les autres paramètres de la fermentation.*

Mots-clés. *Additifs végétaux – Méthanogenèse – Fermentation ruminale.*

I – Introduction

Plant secondary metabolites are a group of chemicals present in plants that are not involved in the primary biochemical processes of plant growth and reproduction but protect the plants against insect predation or grazing by herbivores. Several plant secondary metabolites have shown antimicrobial activity (Cowan, 1999), so they have been suggested as alternative additives to be used in animal feeding (Greathead, 2003), as they can modify ruminal fermentation to enhance the efficiency of utilization of feed energy and decrease methane emissions (García-González *et al.*, 2006).

Ruminants are major contributors to biogenic methane formation. It has been estimated that preventing methane formation from ruminants would stabilise atmospheric methane concentrations and improve animal performance (Johnson and Johnson, 1995). Plant extracts, with high concentrations of secondary compounds are good candidates for reducing ruminal methanogenesis (Teferedegne, 2000) since they are available in large numbers and considered natural products and authorised for human consumption. It is known that some plant extracts that have high flavonoid or tannin contents decrease methane production and induce extensive stimulation of microbial metabolism (Woodward *et al.*, 2001; Broudiscou *et al.*, 2002) although their effectiveness varies depending on the source, type and content of secondary metabolite (Patra *et al.*, 2006).

The six plants used in the present experiment had been identified as potential anti-methanogenic candidates in a previous screening assay in which a collection of 450 plant species had been tested (EC Project "Rumen-up", QLK5-CT-2001-00992, http://www.rowett.ac.uk/rumen_up). These plants were observed to reduce methane production *in vitro* and the objective of this current study was to determine the consistency of this response.

II – Materials and methods

After collection, plants were freeze-dried, ground to pass through a 1 mm sieve, and stored in tightly closed glass jars in a dry, dark and cool place. Candidates for anti-methane effects were selected on the basis of a previous screening assay when they caused a decrease in methane production by more than 20% and had no negative effects on digestibility, total gas production, total volatile fatty acids (VFA) production and propionate to acetate ratio. Six candidates were selected: *Carduus pycnocephalus*, *Populus tremula*, *Prunus avium*, *Quercus robur*, *Rheum nobile* and *Salix caprea*.

In vitro batch cultures of mixed ruminal microorganisms were used to test the effects of plant samples. Three adult rumen cannulated sheep fed alfalfa hay were used as donors for rumen fluid, which was collected before morning feeding, strained through four layers of cheesecloth and kept under a flushing of CO_2. The substrate used for the batch cultures was a mixture of alfalfa hay, grass hay and barley (proportions of 500, 400 and 100 g/kg, respectively) whose chemical composition was as follows (per kg DM): 921 g organic matter, 450 g neutral detergent fibre and 133 g crude protein. Incubations were carried out in 120 ml serum bottles [500 mg DM of substrate, 50 mg DM of each plant, 10 ml strained rumen fluid and 40 ml medium (Menke and Steingass, 1988)]. The bottles were sealed and placed in an incubator at 39°C. Four replicates per plant were incubated, and blank (no substrate and no plant) and controls (550 mg DM of substrate without any plant) were incubated in each of the four incubations carried out.

After 24 h of incubation, accumulated head space pressures and gas volumes were measured. A sample of the gas was transferred to a 10 ml vacuum tube for methane analysis by gas chromatography. Then, fermentation was stopped by swirling the bottles in ice. Each bottle was opened to measure pH in the incubation medium and a sample of supernatant was taken for volatile fatty acids analysis by gas chromatography. All contents remaining in the bottle were filtered, dried and weighed and a sample extracted with neutral detergent solution (van Soest *et al.*, 1991). Fermentation efficiency was calculated as described by Blummel *et al.* (1997).

Results were subjected to one-way analysis of variance with plant sample as the treatment factor and incubation run as a blocking factor. Multiple comparisons of means were performed using the Duncan test (Steel and Torrie, 1980).

III – Results and discussion

Methane production, DM digestibility, total gas production, fermentation efficiency, VFA production and pH after 24 h of incubation are reported in Table 1. All tested plants reduced methane production ($P < 0.001$) in relation to the control (no plant added), and gas production, pH and fermentation efficiency were not affected ($P > 0.05$) by any of the plants. *P. avium* and *Q. robur* caused a decrease in propionate production and in propionate to acetate ratio ($P < 0.001$), whereas *S. caprea* reduced propionate production ($P < 0.001$). *R. nobile* increased DM digestibility ($P < 0.05$), VFA production ($P < 0.001$) and propionate to acetate ratio ($P < 0.001$).

Table 1. Effects of *Carduus pycnocephalus*, *Populus tremula*, *Prunus avium*, *Quercus robur*, *Rheum nobile* and *Salix caprea* on rumen fermentation parameters *in vitro* after 24 h incubation

	Control	*Carduus*	*Populus*	*Prunus*	*Quercus*	*Rheum*	*Salix*	SED†	P
Methane (mmol/g DM incubated)	1.094^{a}	0.999^{b}	1.001^{b}	1.043^{b}	0.991^{b}	0.941^{c}	1.041^{b}	0.0247	<0.001
Methane (mmol/g DM digested)	1.664^{a}	1.500^{b}	1.495^{b}	1.544^{b}	1.502^{b}	1.388^{c}	1.566^{b}	0.0390	<0.001
Methane (mmol/mol gas)	174^{a}	163^{bc}	160^{c}	163^{bc}	161^{bc}	151^{d}	168^{ab}	3.3	<0.001
DM digestibility	0.662^{b}	0.666^{b}	0.671^{ab}	0.673^{ab}	0.664^{b}	0.678^{a}	0.668^{ab}	0.0054	0.036
Gas (mmol/g DM incubated)	6.31^{ab}	6.14^{b}	6.23^{ab}	6.40^{a}	6.13^{b}	6.22^{b}	6.20^{b}	0.084	0.034
Fermentation efficiency (mg DM digested/ml gas)	4.14	4.29	4.28	4.15	4.29	4.32	4.26	0.071	0.130
Propionate:acetate ratio	0.295^{b}	0.295^{b}	0.290^{bc}	0.283^{cd}	0.278^{d}	0.316^{a}	0.288^{bc}	0.036	<0.001
VFA (mmol/g DM incubated)	4.95^{b}	4.99^{b}	4.90^{b}	5.01^{b}	4.92^{b}	5.16^{a}	4.90^{b}	0.069	<0.001
Propionate (mmol/mol VFA)	206^{b}	206^{b}	204^{bc}	201^{cd}	198^{d}	216^{a}	202^{c}	1.7	<0.001
pH	6.68	6.60	6.64	6.65	6.60	6.59	6.65	0.020	0.466

†SED = standard error of the difference.
^{a,b,c,d}Means with different superscript differ significantly ($P < 0.05$).

Some of the methane inhibitors may have adverse effects on ruminal metabolism or animal physiology, such as reducing digestibility (Beauchemin and McGinn, 2006). However, some plants decrease methane production and stimulate microbial metabolism, increasing degradability of crude protein and cell wall constituents as well as the yield of microbial biomass (Broudiscou *et al.*, 2002). Lack of effect on nutrient degradation in response to some plant extracts that reduced methane production has been reported by Sliwinski *et al.* (2002). In the present experiment, most of the plant samples did not modify any of the parameters studied other than methane production, so they may have the potential to be used to improve the ruminal fermentation profile.

Methane production has been reported to decrease in response to secondary compounds from medicinal plants. Phenolic compounds, such as tannins, reduced rumen methanogenesis in sheep and cattle (Woodward *et al.*, 2001), probably due to both direct effects on methanogen activity and indirect effects on fibre digestion. Saponins decreased methanogenesis *in vitro* (Lila *et al.*, 2003); with a mode of action related probably to their anti-protozoal activity (Newbold and Rode, 2006), even though changes in methane production were not always related to similar variations in

protozoa numbers (Patra *et al.*, 2006). Flavonoids, and their degradation products, have been demonstrated to modify the microbial metabolism in the rumen (Broudiscou and Lassalas, 2000).

Some of these plant secondary compounds with anti-methane activity have been detected in the plant species evaluated in the current study. Several different secondary metabolites have been isolated from *C. pycnocephalus*, including flavonoids (Bain and Desrochers, 1988), hexadecanoic acid (Esmaeili *et al.*, 2005), and a flavone glycoside with antimicrobial activity (El-Lakany *et al.*, 1997). *R. nobile* accumulates a substantial quantity of flavonoids (Iwashina *et al.*, 2004). This plant species contains other bioactive compounds such as anthraquinones with anti-bacterial and anti-viral properties (Babu *et al.*, 2003). Nitrogen-containing secondary compounds, such as non-protein amino acids, or cyanogenic glycosides have been isolated from *P. avium* (Harborne, 1991). Aspens (*Populus* spp.) and willows (*Salix* spp.) appear to contain structurally related secondary phenolics and isoprenoids, which are generally known to be their defence against herbivores (Palo, 1984), as well as glycosides, such as salicin (Rowell-Rahier, 1984). Lignans, phenolic compounds that have been shown to inhibit fungal growth, have been reported in *Salix* spp. and *Quercus* spp. (Gottlieb and Yoshida, 1989). Tannins are secondary metabolites present in *S. caprea* (Juntheikki, 1996), while *Q. robur* has been reported to contain flavonoids, such as quercitin, terpenoids (Harborne, 1991), triterpene saponins and condensed tannins (Arramon *et al.*, 2002).

The interspecific differences between plant samples make it difficult to relate observed effects to the presence of particular phytochemicals, so the mode of action of some of the plants selected in this study has not been elucidated. In conclusion, among the plant species evaluated in the current study, *Rheum nobile* was particularly promising since it reduced methane production by the greatest amount and most consistently of the plants tested (–16% in relation to the control) and also enhanced ruminal fermentation.

References

Arramon G., Saucier C., Colombani D. and Glories Y., 2002. Identification of triterpene saponins in *Quercus robur* L. and *Q. petraea* Liebl heartwood by LC-ESI/MS and NMR. In: *Phytochem. Anal.*, 13. p. 305-310.

Babu K.S., Srinivas P.V., Praveen B., Kishore K.H., Murty U.S. and Rao J.M., 2003. Antimicrobial constituents from the rhizomes of *Rheum emodi*. In: *Phytochemistry*, 62. p. 203-207.

Bain J.F. and Desrochers A.M., 1988. Flavonoids of *Carduus-Nutans* and *Carduus-Acanthoides*. In: *Biochem. Syst. Ecol.*, 16. p. 265-268.

Beauchemin K.A. and McGinn S.M., 2006. Effects of various feed additives on the methane emissions from beef cattle. In: *Int. Congr. Ser.*, 1293. p. 152-155.

Blummel M., Makkar H.P.S. and Becker K., 1997. *In vitro* gas production: A technique revisited. In: *J. Anim. Physiol. Anim. Nutr.*, 77. p. 24-34.

Broudiscou L.P. and Lassalas B., 2000. Effects of *Lavandula officinalis* and *Equisetum arvense* dry extracts and isoquercitrin on the fermentation of diets varying in forage contents by rumen microorganisms in batch culture. In: *Reprod. Nutr. Dev.*, 40. p. 431-440.

Broudiscou L.P., Papon Y. and Broudiscou A.F., 2002. Effects of dry plant extracts on feed degradation and the production of rumen microbial biomass in a dual outflow fermenter. In: *Anim. Feed Sci. Technol.*, 101. p. 183-189.

Cowan M.M., 1999. Plant products as antimicrobial agents. In: *Clin. Microbiol. Rev.*, 12. p. 564-582.

El-Lakany A.M., Abdel-Kader M.S., Hammoda H.M., Ghazy N.M. and Mahmoud Z.F., 1997. A new flavone glycoside with antimicrobial activity from *Carduus pycnocephalus* L. In: *Pharmazie*, 52. p. 78-79.

Esmaeili A., Rustaiyan A. and Nadimi M., 2005. Volatile constituents of *Centaurea depressa* MB and *Carduus pycnocephalus* L. two Compositae herbs growing wild in Iran. In: *J. Essent. Oil Res.*, 17. p. 539-541.

García-González R., López S., Fernández M. and González J.S., 2006. Effects of the addition of some medicinal plants on methane production in a rumen simulating fermenter (RUSITEC). In: *Int. Congr. Ser.*, 1293. p. 172-175.

Gottlieb O.O. and Yoshida M., 1989. Lignans. In: Rowe J.W. (ed.). *Natural Products of Woody Plants*. Berlin, Germany: Springer-Verlag. p. 349-511.

Greathead H., 2003. Plants and plant extracts for improving animal productivity. In: *Proc. Nutr. Soc.*, 62. p. 279-290.

Harborne J.B., 1991. The chemical basis of plant defense. In: Palo R.T. and Robbins C.T. (eds). *Plant Defenses against Mammalian Herbivory*. New York, USA: CRC Press. p. 45-59.

Iwashina T., Omori Y., Kitajima J., Akiyama S., Suzuki T. and Ohba H., 2004. Flavonoids in translucent bracts of the Himalayan *Rheum nobile* (Polygonaceae) as ultraviolet shields. In: *J. Plant Res.*, 117. p. 101-107.

Johnson K.A. and Johnson D.E., 1995. Methane emissions from cattle. In: *J. Anim. Sci.*, 73. p. 2483-2492.

Juntheikki M.R., 1996. Comparison of tannin-binding proteins in saliva of Scandinavian and North American moose (*Alces alces*). In: *Biochem. Syst. Ecol.*, 24. p. 595-601.

Lila Z.A., Mohammed N., Kanda S., Kamada T. and Itabashi H., 2003. Effect of sarsaponin on ruminal fermentation with particular reference to methane production *in vitro*. In: *J. Dairy Sci.*, 86. p. 3330-3336.

Menke K.H. and Steingass H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. In: *Anim. Res. Dev.*, 28. p. 7-55.

Newbold C.J. and Rode L.M., 2006. Dietary additives to control methanogenesis in the rumen. In: *Int. Congr. Ser.*, 1293. p. 138-147.

Palo R.T., 1984. Distribution of birch (*Betula* spp.), willow (*Salix* spp.), and poplar (*Populus* spp.) secondary metabolites and their potential role as chemical defense against herbivores. In: *J. Chem. Ecol.*, 10. p. 499-520.

Patra A.K., Kamra D.N. and Agarwal N., 2006. Effect of spices on rumen fermentation, methanogenesis and protozoa counts in *in vitro* gas production test. In: *Int. Congr. Ser.*, 1293. p. 176-179.

Rowell-Rahier M., 1984. The presence or absence of phenolglycosides in *Salix* (Salicaceae) leaves and the level of dietary specialisation of some of their herbivorous insects. In: *Oecologia*, 62. p. 26-30.

Sliwinski B.J., Soliva C.R., Machmuller A. and Kreuzer M., 2002. Efficacy of plant extracts rich in secondary constituents to modify rumen fermentation. In: *Anim. Feed Sci. Technol.*, 101. p. 101-114.

Steel R.G.D. and Torrie J.H., 1980. *Principles and Procedures of Statistics*, 2nd edn. New York, USA: McGraw-Hill.

Teferedegne B., 2000. New perspectives on the use of tropical plants to improve ruminant nutrition. In: *Proc. Nutr. Soc.*, 59. p. 209-214.

van Soest P.J., Robertson J.B. and Lewis B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. In: *J. Dairy Sci.*, 74. p. 3583-3597.

Woodward S.L., Waghorn G.C., Ulyatt M.J. and Lassey K.R., 2001. Early indication that feeding lotus will reduce methane emission from ruminants. In: *Proc. N. Z. Soc. Anim. Prod.*, 61. p. 23-26.