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SUMMARY – Tannins are naturally occurring polyphenols found in many forage plants that reduce their nutritional value through an inhibitory effect on ruminal and intestinal functions. Daily supplementation with polyethylene glycol (PEG) has been proven to efficiently neutralise the negative effect of tannins. A grazing experiment was undertaken to evaluate the effect of PEG supplementation on animal performance and milk fatty acid composition of Sarda sheep grazing sulla (*Hedysarum coronarium*). Twenty-four late-lactating sheep (12 per group), were paired and split into two groups: group CON daily dosed by a quenching gun with 200 ml of water, and group PEG, dosed with 200 ml of a 50/50 water solution of PEG. The sheep rotationally grazed two 0.8 ha plots of sulla. Milk yield tended (P<0.13) to be increased by PEG. Milk fat and protein contents were not affected by the treatment. The CLA (C18:2 *c-9*, *t-11*) and vaccenic acid (C18:1 *t-11*) contents in milk fat were higher on average by 40% for PEG than CON group. This can be explained by a higher biohydrogenation activity of ruminal bacteria in the PEG group, due to the partial inactivation of tannins. Actually both linoleic (C18:2 *c-9 c-12)* and linolenic (C18:3 *c-9 c-12 c-15*) fatty acid were lower (P<0.05) in milk from PEG than CON group (-12% and -30% for linoleic and linolenic acids, respectively).

Keywords: PEG, milk, dairy sheep, fatty acid composition, CLA.

RESUME – *"Effet de l'administration de polyéthylène glycol (PEG) sur la composition du lait en acides gras pour des ovins sur un pâturage de Sulla (*Hedysarum coronarium*)". Les tannins sont des polyphénols que l'on trouve à l'état naturel dans de nombreux fourrages, qui réduisent la valeur nutritive et ont un effet inhibiteur sur les fonctions digestives des ruminants. Une complémentation quotidienne de polyéthylène glycol a été expérimentée pour neutraliser ces effets négatifs. Un essai au pâturage sur* Hedysarum coronarium *avec des brebis laitières a été conduit afin d'évaluer l'effet de la complémentation avec PEG sur les performances des animaux et sur la composition en acides gras du lait. Vingt-quatre brebis en fin de lactation (12 par groupe) ont été divisées en deux groupes : un groupe témoin (CON) dosé chaque jour avec 200 ml d'eau, et un groupe supplémenté (PEG) avec 200 ml d'une solution d'eau et PEG (50/50). La production du lait tend (P<0,13) à augmenter par l'administration du PEG. La composition en matières grasses, en protéines du lait n'a pas été affectée. La composition en CLA (C18:2 c-9, t-11) et en acide trans-vaccénique (C18:1 t-11) dans la matière grasse du lait est en moyenne plus élevée (+40%) dans le groupe PEG. Ceci peut s'expliquer par une activité de biohydrogénation des bactéries du rumen dans le groupe PEG due à une inactivation partielle des tannins. La composition en acides gras linoléique (C18:2 c-9 c-12) et linolénique (C18:3 c-9 c-12 c-15) du lait de brebis complémentées avec du PEG est inférieure (P<0,05) à celle du groupe témoin (-12% et -30% pour les acides linoléique et linolénique, respectivement).*

Mots-clés : *PEG, lait, brebis laitières, composition en acides gras, CLA.*

Introduction

Polyphenols are broadly distributed in the plant kingdom and are the most abundant secondary metabolites found in plants (Shi *et al*., 2003). A wide range of secondary compounds have been implicated as anti-nutritional components of animal feed including several types of phenolic compounds such as tannins. Tannins have both beneficial and adverse effects. Beneficial effects in ruminants include bloat suppression (Jones and Mangan, 1977) and protection of dietary protein from rumen degradation which results in increased amino acid absorption and utilization by ruminant animals (Beck and Reed, 2001). Moreover tannins can also protect them against gastrointestinal parasitism (Paolini *et al*., 2002). Adverse effects of tannins includes: (i) lower intake and digestibility; (ii) inhibition of digestive enzymes; (iii) increased loss of endogenous protein; and (iv) systemic effects

as a result of the uptake of hydrolysable tannins degradation products from the digestive tract. In tropical regions also a lethal effect of tannins, ingested by animals, has been reported (Van Hoven, 1984). Lance and David (2001) pointed out that animal performance is reduced when tannins exceeded 5% of DM intake, and Hegarty *et al*. (1985) observed that both condensed and hydrolysable tannins can exert toxic effects.

The tannin content in herbaceous forages is usually low with the exception of some legumes like sulla (*Hedysarum coronarium* L.). Results from Molle *et al*. (2003) showed that condensed tannin levels in sulla ranged between 2% and 4% of DM, depending on phenological phase. However other studies under different environmental conditions highlighted that condensed tannins in sulla forage can be higher than 5% (Niezen *et al*., 1995). Makkar (2001) indicates several options to alleviate the negative effect of tannins (wood ash, storage, drying, chemicals solid-state fermentation and administration of polyethylene glycol (PEG). As a matter of fact the use of polyethylene glycol (PEG) is the only approach that seems to be effective to reduce the negative effect of tannins in animal nutrition as reported by recent works in goats (Decandia *et al*., 2000b; Titus *et al*., 2001, Ben Salem *et al.,* 2003, Yáñez Ruiz *et al.,* 2004), and in sheep (Villalba and Provenza, 2002). The aim of this work was to evaluate the effect of PEG supplementation on animal performance and milk fatty acid composition in sheep grazing sulla.

Materials and methods

The trial was conducted during spring 2004 (April-May, flowering stage of pasture) at the Bonassai research farm. Two experimental plots (8000 m^2) of sulla (cultivar Grimaldi) were sown in October 2003 and subsequently rotationally grazed by dairy sheep (grazing period = 14 days). Twenty-four lactating sheep, homogeneous for live weight and milk yield were randomly allocated to the following treatments: control (CON) dosed daily with 200 ml of water and PEG dosed with 200 ml of a 50/50 water solution of polyethylene glycol (at milking). The groups grazed as a flock during the whole day with the exception of milking (twice a day). No supplement was offered. Hand-plucked samples of grazed forage species were taken to measure DM, CP, EE, NDF, (Martillotti *et al.*, 1987), tannic phenols by the Folin Ciocalteau method and fatty acid composition (Christie, 1989; Chin *et al*., 1992).

Individual milk yield and composition (fat, protein, lactose and urea) was recorded fortnightly. Milk fatty acid profile was determined by gas chromatography (Murphy *at al*., 1990; Chin *et al*., 1992). All data were analysed by GLM with sampling data, treatments and their interaction as fixed effects.

Results and discussion

The chemical composition of sulla is characterised by a high level of CP (22.92% DM), a medium content of EE (2.79% of DM) and a medium content of tannic phenols (2.66% of DM) very close to that reported by Molle *et al*. (2003) and Cabiddu *et al*. (2003) at the same phenological phase. Also the herbage fatty acids composition confirmed that sulla is very rich in linolenic acid (11.26 g/kg of DM) while linoleic acid content is low (1.96 g/kg of DM) as reported by Cabiddu *et al*. (2003). No effect of PEG treatment ($P<0.13$) was observed either on milk yield and milk composition except for milk urea content (Table 1) that was higher in PEG group (52.09 *vs* 45.06 mg/100 ml; $P<0.05$) as a consequence of tannin inactivation. It is well-known that PEG, forming complexes with condensed tannins, involves an increase of ammonia in the rumen (Barry, 1989) and urea in blood (Núñez-Hernandez *et al*., 1991). This can be due to an increase of nutrient availability (in particular CP) and a reduction of microbial inhibition. These findings are in agreement with an increase of CP apparent digestibility found in goats stall-fed lentisk branches (Decandia *et al*., 2000b) and of milk urea in browsing goats (Decandia *et al*., 2000a) as a consequence of PEG dosing. No effect of the period was observed in milk yield and milk composition except for urea content. Milk fatty acid composition was affected by PEG supplementation. In particular vaccenic acid and CLA were 40% higher ($P<0.05$) in PEG than in control group whereas PUFA were higher in CON than in PEG group (Table 2). This could be due to a higher biohydrogenation at rumen level in PEG group because of the effect of tannins on microbial activity was decreased.

Table 1. Milk yield and composition of lactating dairy sheep grazing sulla without (CON) or with (PEG) PEG supplementation

		Treatment group		Sem	Probability level†		
		CON	PEG		tr	per	tr*per
Milk yield	ml/head day	1217	1348	49.74	ns	ns	ns
Milk fat	%	6.75	6.56	0.14	ns	ns	ns
Milk protein	%	5.84	5.84	0.07	ns	ns	ns
Milk lactose	%	4.77	4.69	0.03	ns	ns	ns
Milk urea	mg/100 ml	45.06	52.09	0.97	**	**	ns

†Effects of treatment (tr), date of sampling (per) and their interaction; ns: P>0.10; **= P<0.001.

Table 2. Milk fatty acids composition of lactating dairy sheep grazing sulla without (CON) or with (PEG) PEG supplementation

		Treatment group		Sem	Probability level†		
		CON	PEG		tr	per	tr*per
Vaccenic acid	mg/g of fat	28.35	39.64	1.23	**	**	ns
CLA	mg/g of fat	12.61	17.54	0.55	**	**	*
Linoleic acid	mg/g of fat	21.21	18.79	0.53	**	**	ns
Linolenic acid	mg/g of fat	40.02	28.55	1.28	**	**	ns
PUFA	mg/g of fat	73.84	64.87	1.50	*	**	ns

†Effects of treatment (tr), date of sampling (per) and their interaction; ns: P>0.10; *= P<0.05; **=P<0.001.
CLA = C18:2 c-9, t-11; PUFA = polyunsaturated fatty acids.

Overall, these results are in agreement with those of Getachew *et al*. (2001) who found an increased of short chain fatty acids content as a consequence of PEG addition on *in vitro* fermentation system of tannin-rich browses. In our case no relationship was found between urea and CLA level in milk to explain this effect.

A significant decrease in milk CLA content was observed during the period, in particular in CON group as observed by Piredda *et al.* (2002). Therefore PEG administration can mitigate the CLA decrease in milk of sulla grazing sheep during late spring, that is observed usually in association with pasture maturation.

Conclusion

The results obtained in the present study confirm the influence of tannins on nutritional characteristics of sulla and indicate the possibility of improving its nutritional value by inactivating tannins. In fact the inactivation of tannins with PEG supplementation can modulate ruminal biohydrogenation and modify lipid metabolism, enhancing the nutraceuticals properties of dairy products with reference to vaccenic acid and CLA.

On the other hand, the milk of the control group is also characterised by high nutritional value due to its higher content of PUFA in comparison with PEG group, in particular of linolenic acid, an ω3-fatty acids which plays an important role on human health.

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