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Effect of the administration of PEG on milk yield and composition in sheep browsing a tanniniferous fodder tree

H. Ammar*, S. López**, F. Opsi**, R. Bodas**, J.S. González and S. Andrés**

*Ecole Supérieure d'Agriculture de Mograne, IRESA, Mograne-Zaghouan 1121 (Tunisia)

**Instituto de Ganadería de Montaña, CSIC-Universidad de León, 24346 León (Spain)

Abstract. The objective of this study was to examine how the administration of a tannin-neutralising agent (polyethylene glycol, PEG) to lactating sheep browsing a tanniniferous fodder tree could affect the ewes' performance in terms of milk yield and composition. Forty lactating sheep of a local Tunisian breed (Barbarine) were used. Sheep were browsing the fodder tree *Acacia cyanophylla* Lindl. as their basal diet, supplemented with grass hay and concentrate. Animals were allocated in two experimental groups, namely control and PEG. Sheep of the PEG group received an oral dose of PEG 4000 (20 g/d). Milk yield was recorded the first day of treatment (P1, corresponding on average to 77 days of lactation) and then at 41 and 75 days thereafter (P2 and P3, respectively), and samples of milk were collected to determine fat, protein and urea contents. Milk yield at P1 and P3 was not affected by the administration of PEG, with average values for control and PEG groups of 799 and 854 g (SEM 111.9) at P1, and 476 and 468 g (SEM 25.9) at P3. There were differences ($P = 0.007$) between experimental groups in milk yield at P2, with average values of 709 and 884 g (SEM 43.4) for control and PEG ewes, respectively. Milk fat was higher at P1 and P3 (average fat content 105 g/kg) than at P2 (75 g/kg), and there were no effects ($P > 0.10$) of PEG supplementation on this trait. Milk protein was similar through the lactation with average protein content of 58 g/kg, and was not affected by PEG. There was not a significant effect of PEG on urea concentration in milk, which showed a noticeable variation through lactation with lowest values at P2. In conclusion, the administration of PEG to lactating sheep browsing a tanniniferous fodder tree had no effects on milk composition, but an increase in milk yield in mid lactation was observed in response to PEG, indicating that animals could benefit from higher supply of feed protein owing to the binding of *Acacia* tannins by this compound.

Key words. Sheep – Milk – PEG – Tannins.

Effet de l'administration du PEG sur la production et la composition du lait des brebis pâturent sur parcours d'arbuste riche en tanins

Résumé. L'objectif de ce travail est de montrer si l'addition d'un agent neutralisant des tannins (PEG) à des brebis en lactation pâturent sur maquis d'arbustes fourragers pourrait affecter leurs performances laitières en termes de rendement et de composition du lait. Quarante brebis de race Barbarine en lactation ont été utilisées. Toutes les brebis ont été mises sur un parcours d'*Acacia cyanophylla* Lindl. en tant qu'aliment de base, et supplémentées par le foin d'herbe et le concentré. Les animaux ont été répartis en deux groupes expérimentaux nommés control et PEG. Par voie orale, les brebis du groupe PEG ont reçue une dose PEG 4000 (20 g/j). La production laitière a été mesurée le premier jour du traitement (P1, correspondant en moyenne au 77^{ème} jours de lactation) puis au 41^{ème} et 75^{ème} jours suivants (P2 et P3, respectivement), des échantillons du lait ont été collectés pour déterminer les contenus en protéine, matière grasse et urée. La production laitière mesurée à P1 et P3 n'était pas affectée par l'administration du PEG, étant en moyenne, pour les deux groupes control et PEG, 799 et 854 g (ESM 111,9) à P1, et 476 et 468 g (SEM 25,9) à P3. Cependant, la production laitière enregistrée à P2 a été significativement différente ($P = 0,007$) entre les deux groupes, avec une moyenne de 709 et 884 g (ESM 43,4) pour le groupe contrôle et PEG, respectivement. La matière grasse était plus élevée à P1 et P3 (en moyenne 105 g/kg) qu'à P2 (75 g/kg), sans qu'il y ait un effet significatif ($P > 0,10$) du PEG sur ce paramètre. Durant toute la lactation, le contenu protéique du lait était constant (58 g/kg) et n'était pas affecté par l'addition du PEG. De même, la concentration du lait en urée n'était pas affectée suite à l'administration du PEG; cependant elle a marqué une variation notable tout au long de la lactation en enregistrant la valeur la plus faible à P2.

En conclusion, l'administration du PEG à des brebis en lactation pâturant sur parcours d'Acacia cyanophylla n'a pas d'effet sur la composition du lait, mais une augmentation de la production laitière au milieu de la lactation a été observée comme réponse au PEG, indiquant que les animaux pourraient bénéficier d'un ajout supplémentaire de protéine alimentaire grâce à la désactivation des tannins de l'Acacia par cet agent neutralisant.

Mots-clés. Ovin – Lait – PEG – Tannins.

I – Introduction

In Tunisia, a major constraint to the performance of grazing ruminants is the scarcity of high quality pastures, particularly during severe droughts. The situation is particularly worse at late pregnancy and during lactation, when animal requirements are higher. During the last decade, about 600,000 ha have been cultivated with shrubby vegetation to combat desertification, mitigating the effects of droughts, allowing soil fixation and enhancing the restoration of the vegetation and the rehabilitation of rangelands.

Although some of these browse plants are rich in protein (Ammar *et al.*, 2004, 2005), most species contain variable amounts of structurally diverse secondary compounds such as tannins and other compounds with antinutritional properties. Tannins are defined as naturally occurring polyphenolic compounds of high molecular weight that form complexes with proteins and carbohydrates. Polyethylene glycol (PEG), a tanning binding agent, has proven efficient in neutralizing tannins (Jones and Mangan, 1977), hence improving the efficiency of utilization of several tanniferous species by animals (Makkar, 2003a).

Despite the fact that negative effects of tannins on milk production and composition have been reported in cattle (Rémond, 1985; DePeters and Cant, 1992) and goats (Morand-Fehr *et al.*, 1991), less information is available regarding the effects of tannins on milk composition in dairy ewes (Bocquier and Caja, 1993; Pulina *et al.*, 2006). In Tunisia, most of the work carried out on shrub species such as *Acacia cyanophylla* have focused on their effects on animal behavior and some metabolic aspects. However, little is known on effects of tannins on milk production in sheep. The current study aimed to evaluating the effect of feeding browse of *A. cyanophylla* on milk yield and composition in ewes at different stages of lactation, examining the response when PEG was administered to neutralize tannin effects.

II – Material and methods

1. Animals

Forty lactating sheep (average age 5.4 years SE 0.32; average weight 43.5 kg SE 1.25) of a local Tunisian breed (Barbarine) were used in the study. Animals belonged to a private flock raised in Zaghouan (Tunisia) and registered in the official quantitative milk recording programme of the Tunisian Office de l'Élevage et des Pâturages. Lambing was in September, and weaning was on average 152 days after lambing at the end of the experiment. Sheep were browsing the *Acacia cyanophylla* Lindl. and herbaceous vegetation, and then supplemented with grass hay (0.3 kg) and concentrate (0.4 kg). Concentrate was offered in two equal meals at 08:00 h and 16:00 h. After the morning meal sheep were allowed to browse *Acacia* fodder for approximately 5 h every day, and then housed in the sheepfold where hay and concentrate were distributed. During the trial, animals had free access to clean water. Lambs remained always with their dams, even when animals were in the *Acacia* plantation.

Application of experimental treatments and measurements started at two and half months after lambing and lasted for 75 days. Ewes were randomly allocated in two experimental groups (considering live weight and time after lambing) namely PEG and control. PEG ewes received an oral dose (20 g/day) of polyethylene glycol 4000 (through the administration of 40 ml of a solution of 500 g PEG/liter), whereas sheep in the control group received daily 40 ml of distilled water. PEG or distilled water (in control ewes) were dosed in the morning just before ewes were allowed to graze in the *Acacia* plantation. Milk yield was recorded the first day of treatment (P1, corresponding on average to 77 days after lambing) and then at 41 (P2) and 75 (P3) days thereafter. In milking days, ewes were maintained into the sheepfold, and the lambs were separated from their dams. At 08.30 h each ewe was injected intravenously one IU of oxytocin in 1.0 ml saline (Oxytocin EA, Ethical Agents Ltd, Auckland, New Zealand) (Ricoardeau *et al.*, 1960). Then, ewes were handily milked and the milk collected was discarded. Two hours later, ewes were milked again as previously described to measure the amount of milk produced in this period. After milking lambs joined their dams and the flock was allowed to graze in the *Acacia* cultivated area.

During milking, a sample of 20 ml of milk was collected in plastic flasks containing 1 mg of potassium dichromate in order to avoid any possible fermentation. Milk samples were then delivered immediately to the laboratory (Sidi Thabet, Tunisia) (fat, protein and urea contents) using an integrated milk analyser (Combifoss 5300, Foss Electric, Denmark). Daily milk yield was calculated from the milk production recorded over a period of 2 hours.

2. Chemical analysis

Dry matter (DM, method ID 934.01), ash (method ID 942.05) and crude protein (CP, method ID 984.13) contents of leaves and young twigs of *A. cyanophylla* were determined following the methods of AOAC (1999). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) contents were determined with the ANKOM fibre analyzer (Ammar *et al.*, 1999) using the reagents described by Van Soest *et al.* (1991). Total tannins (as tannic acid equivalent) and condensed tannins (as leucocyanidin equivalent) were determined in acacia fodder following the methods described by Makkar (2003b). All chemical analyses of browse samples were conducted at the University of León (Spain).

3. Statistical analysis

Statistical differences between experimental groups (control vs PEG) in milk production, fat, protein and urea contents were evaluated at each experimental period by one way analysis of variance (Steel and Torrie, 1980).

III – Results and discussion

Chemical composition of *A. cyanophylla* (leaves and young twigs), hay and concentrate is shown in Table 1. Cell wall components were higher in hay and stems of *A. cyanophylla* than in leaves and in concentrate, whereas an opposite trend was observed for CP content. Leguminous shrubs and trees have been used as feedstuffs for livestock in many regions of the world, mainly because of their high protein contents throughout the year (Ammar *et al.*, 2004) that can be attributed to the ability of these plants to fix atmospheric nitrogen. Digestive utilization of nitrogenous compounds depends on the presence of tannins and other phenolic compounds (Woodward and Reed, 1989; Rittner and Reed, 1992), commonly present in some browse species.

Acacia fodder material contained 44 g total tannins/kg DM and 72 g condensed tannins/kg DM. Moujahed and Kayouli (2004) determined the concentration of condensed tannins in leaves of *A. cyanophylla* to be 42 g/kg DM. These authors observed an improved daily weight gain in lambs

fed on *A. cyanophylla* in response to the administration of PEG. In the present study, PEG was administered to dairy ewes to examine effects of *Acacia* tannins on milk chemical composition and yield at different stages of lactation (77, 118 and 152 days) corresponding respectively to 0 (P1), 41 (P2) and 75 (P3) days of PEG treatment.

Table 1. Chemical composition (g/kg dry matter) of different components of the ration

Feed	Dry matter (g/kg)	Ash	Crude protein	NDF	ADF
<i>A. cyanophylla</i> leaves	360	125	144	252	193
<i>A. cyanophylla</i> stems	420	58	69	576	421
Hay	960	111	78	698	416
Concentrate	880	99	152	282	173

Milk composition and daily yield are shown in Table 2. The consumption of *A. cyanophylla* was not associated with any detrimental effects on milk components (protein, fat and urea) in agreement with Molle *et al.* (2009). Condensed tannin contents in *A. cyanophylla* are moderated (40-70 g/kg DM) and at such concentrations, positive effects of condensed tannins on by-pass proteins and milk production may be observed (Wang *et al.*, 1996; Woodward *et al.*, 1999; Min *et al.*, 2003). In contrast, at higher concentrations (CT>70 g/kg DM), negative effects on protein digestibility become apparent in ruminants (Barry and McNabb, 1999; Min *et al.*, 2003).

Table 2. Milk components and milk yield (g/d) in ewes of control or treated (PEG) group

	P1			P2			P3		
	Control	PEG	SEM	Control	PEG	SEM	Control	PEG	SEM
Protein (g/kg)	59	58	1.6	57	59	0.6	57	57	0.8
Fat (g/kg)	109	104	3.0	75	75	1.5	103	104	7.1
Milk urea (mg/dl)	117	113	3.8	62	68	5.8	122	113	5.4
Milk yield	799	854	111.9	709 ^b	884 ^a	43.4	476	468	25.9

Means with different superscript letters within a row and at the same treatment day indicate differences at $P < 0.05$.

Milk protein content remained within a narrow range (between 57 and 59 g/kg) through the lactation. The highest fat contents in milk (>100 g/kg) were recorded at the beginning (P1) and the end (P3) of the experiment, whereas at P2 milk fat content was noticeably lower (75 g/kg). A number of factors can affect quality of milk collected in sheep farms and explain the variability of responses of milk composition to ewe's nutrition in practice (Frayse *et al.*, 1996). It is important to bear in mind that ewes were under a suckling-and-milking management. Milk urea contents were not different between groups in contrast with results reported by Molle *et al.* (2009) showing higher milk urea concentrations in PEG drenched ewes fed sulla, probably due to higher concentrations of rumen ammonia in these animals, suggesting a change in feed protein utilization.

Regarding milk yield, a decline in milk production with lactation was observed. There were significant differences ($P < 0.05$) between experimental groups at 41 days of PEG application (P2), so that yield was 25% higher in PEG ewes compared to control ewes. The positive effect of PEG supplementation on milk yield confirmed previous findings by Decandia *et al.* (2000a,b, 2008) and Gilboa *et al.* (2000). This would suggest a better utilization of feed protein in PEG drenched ewes.

IV – Conclusion

Administration of PEG to lactating sheep browsing a tanniniferous fodder tree had no effects on milk composition, but an increase in milk yield in mid lactation and after 40 days of administration was observed in response to PEG, indicating that animals could benefit from higher supply of feed protein owing to the binding of *Acacia* tannins by this compound.

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