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Evaluation of the anthelmintic activity of *Acacia cyanophylla* Lindl against gastrointestinal nematodes of sheep: *in vivo* study

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Abstract. The potential use of *Acacia cyanophylla* Lindl. (acacia), a tanniniferous shrub species, as a biologic way to reduce gastro-intestinal parasites was tested in grazing Barbarine lambs. Three groups of 10 lambs each were allocated to one of the following dietary treatment according to a cross-over design: grazing native pasture free of acacia and other woody species (grass group), grazing acacia (acacia group), and grazing acacia after daily oral administration of 40 g polyethylene glycol (PEG mol. weight 4000) (acacia plus PEG-group). PEG, a potent deactivating agent for condensed tannins, was mixed in water (1 kg in 2 l of water) and administered orally. Animals received daily at 8:30 h 300 g concentrate, and were then allowed to graze in the pasture or in the acacia plantation from 10:00 h to 16:00 h. Oaten hay (500 g) was distributed to lambs in the barn at 16:30 h. Each of the three experimental periods lasted 4 weeks. When lambs were on acacia diet they showed a significantly lower ($P<0,05$) mean faecal egg counts (FECs) than those on grass, 849.1 ± 453.6 epg and 1001.8 ± 511.8 epg, respectively. The addition of PEG to the acacia diet resulted in an increase in the daily weight gain and plasmatic uraemia concentrations to levels similar to that in the case of grass.

Keywords. *Acacia cyanophylla* Lindl. – Tannins – Faecal eggs – Barbarine lambs.

Evaluation de l'activité antihelminthique d'*Acacia cyanophylla* Lindl contre les nématodes gastro-intestinaux des ovins : étude in vivo

Résumé. L'utilisation potentielle d'*Acacia cyanophylla* Lindl. (acacia), un arbuste riche en tanins, comme un moyen biologique pour réduire les parasites gastro-intestinaux ont été examinés chez les agneaux de pâturage de race Barbarine. Trois groupes de 10 agneaux chacun ont reçu l'un des régimes alimentaires suivants selon un cross-over design : pâturages naturels dépourvus d'acacia et d'autres espèces ligneuses (groupe herbe), pâturage d'acacia et pâturage d'acacia avec administration orale de 40 g de polyéthylène glycol (PEG). PEG, un agent de désactivation puissant des tanins condensés, a été mélangé dans l'eau (1 kg à 2 l d'eau) et administré par voie orale. Les animaux ont reçu quotidiennement à 8 h:30, 300 g de concentré, et sont ensuite libérés pour paître dans les pâturages ou dans la plantation d'acacia à partir de 10 h:00 à 16 h:00. Du foin (500 g) a été distribué aux agneaux dans l'étable à 16 h:30. Chacune des trois périodes expérimentales a duré 4 semaines. Lorsque les agneaux étaient sur le régime d'acacia, ils ont montré une moyenne du nombre d'œufs fécaux (OPG) significativement ($P<0,05$) plus faible que ceux sur l'herbe, $849,1 \pm 453,6$ OPG et $1001,8 \pm 511,8$ ($p<0,05$) epg, respectivement. L'ajout de PEG à la diète d'acacia a entraîné une augmentation du gain de poids quotidien et des concentrations d'urée plasmatique à des niveaux similaires à celle dans le cas de l'herbe.

Mots-clés. *Acacia cyanophylla* Lindl – Tanins – Nématodes gastrointestinaux – Ovins.

I – Introduction

Numerous studies on the nutritive value of *A. cyanophylla* Lindl. (acacia) revealed the presence of high concentrations of tannins in acacia foliage and showed the negative effect of these secondary compounds, which affect the amount of digestible proteins and growth performance of sheep (Ben Salem *et al.*, 1999). It is now well established that the consumption of herbage and

shrub foliage rich in condensed tannins could interfere, in small ruminants, with the development of gastro-intestinal nematodes resulting subsequently in decreasing faecal egg counts and parasites burdens (Niezen *et al.*, 1998). This anti-nematode effect was observed in sheep and goats given different tanniferous herbage and shrubs, as for instance, sainfoin (Paolini *et al.*, 2003a), and Acacia karoo (Kahiya *et al.*, 2003). The increasing occurrence of anthelmintic resistance and the lack of development of new anthelmintics suggest that tanniferous plants could be considered as a potential strategic alternative for the control of nematodes infection in small ruminants.

II – Material and methods

1. Study area

This experiment was carried out at the National Institute of Agricultural Research of Tunisia (INRAT) experimental centre at Oueslatia (central Tunisia, mean annual rainfall 350 mm). *A. cyanophylla* Lindl. (acacia) plantation was established in this centre in 1990.

2. Experimental design and diets

Thirty weaned Barbarine lambs of 4-5-month old (average live weight 20.8 ± 3.4 kg) were used in this study. The animals were allocated in three groups of 10 animals according to their individual faecal egg counts (FECs). Three weeks before the start of the experiment, the animals received about 500 g oaten hay, 300 g barley grains and grazed daily from 10:00 h to 16:00 h. At the start of the experiment the lambs allocated in three groups were fed for 4 weeks as follows: Group 1 (acacia) and Group 2 [acacia/polyethylene glycol (PEG)] grazing 6 h in acacia plantation, and Group 3 grazing 6 h on a native representative of the area (grass). Lambs grazed together daily from 10:00 h to 16:00 h. Lambs on the acacia/PEG diet, received in addition a polyethylene glycol (PEG 4000) water solution (80 ml containing 40 g PEG) administered orally, daily at 9:00 h. Each one of the 30 experimental animals received 300 g concentrate (80% ground barley grains, 17.5% soyabean meal and 2.5% mineral and vitamin supplement) at 8:30 h and 500 g oaten hay at 16:30 h. Animals were supplied water *ad libitum*. The native grass pasture was composed of gramineous species low in tannins. Both of the acacia plantation and the grass pasture were not grazed for at least 1 year prior to the start of this experiment. Each group of lambs was fed successively on the three diets (acacia, acacia/PEG and grass) according to a cross-over experimental design based on three successive grazing periods of 4 weeks as shown in Table 1.

Table 1. Diets allocated to the three groups of lambs along the three periods of the experiment

Group	Period 1	Period 2	Period 3
1	Acacia	Acacia/PEG	Grass
2	Acacia/PEG	Grass	Acacia
3	Grass	Acacia	Acacia/PEG

3. Measurements

Faecal samples were collected from the rectum 1 day before the start of the experiment, then every week along with the three grazing periods. Two blood samples were collected from each animal at the end of each grazing period by puncturing the jugular vein and allowing blood to flow directly into heparinised Vacutainer® tubes. The first blood sample was immediately centrifuged at 3000 rpm for 15 min then the plasma was collected and stored at -20°C until analyzed. The

second blood sample was used for monitoring the haemoglobin concentrations. Samples of acacia foliage, herbaceous strata, oaten hay and concentrate were taken at the beginning and the end of each grazing period.

4. Laboratory analyses

The coprocultures were done according to the protocol described by Kerboeuf *et al.* (1997). Concentrations of inorganic phosphate in serum were determined for each lamb according to the method used by Paolini *et al.* (2003b). Blood haemoglobin concentrations were determined using an haematological automat (Coulter Counter T540). Total phenols, total tannins and condensed tannins were analysed in feeds as described by Makkar (2003a).

5. Calculation and statistical analysis

The FECs recorded on the three diets were compared using the ANOVA-test by considering exclusively the results of the last 3 weeks passed on each diet. Significant differences between the three diets were detected by the Tukey-test. Correlations between natural log-transformed FECs and blood levels of haemoglobin were analysed on each type of diet using the regression analysis.

III – Results and discussion

At day 0 the mean FECs of the three groups of lambs were similar (1595-1715 epg). At the start of the study, during the first grazing period, a significant decrease in FECs was recorded in the three groups of lambs irrespective to the dietary treatment. When the lambs were fed on acacia and acacia/PEG diets they started to exhibit lower FECs than on grass from the second week of the study, for this reason the overall effects of the three diets on faecal excretion of gastrointestinal nematodes eggs were compared on the basis of the natural log-transformed FECs recorded during the 3 last weeks of the study. The highest FECs were found in the grass group (1001.8 ± 511.8 epg), the acacia diet showed the low-est counts (849.1 ± 453.6 epg) followed by acacia/PEG (863.4 ± 434.6 epg). These differences between the three diets were significant (ANOVA test, $P = 0.005$).



Fig. 1. Evolution of the natural log-transformed mean faecal egg counts (FECs) of gastro-intestinal nematodes in experimental groups. Bar: standard deviation.

When lambs were grazing grass, their counts were significantly higher than on acacia and acacia/PEG diets (Tukey-test, $P = 0.008$ and $P = 0.016$, respectively), however, the FECs recorded on acacia and acacia plus PEG were not significantly different (Tukey-test, $P = 0.965$). When the lambs from Groups 1 and 2 were moved from the acacia and acacia/PEG diets to grass, their FECs were increased in average by 15.8%. In contrast, the transfer of Groups 1 and 3 animals from grass to acacia/PEG or acacia diets lead to an average increase of 31.2% in the FECs (Fig. 1). The same

nematode genera were identified on coprocultures carried out along with the experiment, namely, *Haemonchus*, *Teladorsagia*, *Trichostrongylus* and *Nematodirus*.

Table 2 reports the nutritive characteristics of feeds. Herbaceous vegetation was higher in crude protein than acacia foliage. By contrast, the reverse situation was obtained for total phenols, total tannins and condensed tannins. The amount of acacia intake estimated with the bite counting technique was 385 and 763 g DM/day in acacia and acacia plus PEG groups, respectively. Therefore, the corresponding amount of condensed tannins (expressed as leucocyanidine equivalent) could be estimated to 50.7 and 100.4 g for acacia and acacia plus PEG diets respectively. Lambs on grass or acacia/PEG increased more than those fed on acacia without PEG (Table 3). The values of plas-matic urea and the daily weight gain showed a similar pattern of evolution between diets (Table 3). The mean blood haemoglobin concentrations were significantly different for the three diets (ANOVA-test, $P = 0.004$). When on grass, lambs showed significantly lowest mean haemoglobin concentration ($9.362 \text{ g/dl} \pm 0.978$) than when on acacia and acacia/PEG ($P = 0.017$) diets (Table 3). However, there was no significant difference between the haemoglobin concentrations measured among acacia ($9.955 \text{ g/dl} \pm 0.883$) and acacia plus PEG ($10.007 \text{ g/dl} \pm 0.889$) diets (Table 3).

Table 2. Chemical composition of feeds (g/kg DM, otherwise indicated in the table)

	Acacia foliage	Oaten hay	Concentrate
Dry matter (g/kg)	317	891	905
Organic matter	870	903	925
Crude protein	118	86	171
Total phenols [†]	64.0	3.6	2.7
Total tannins [†]	32.3	0.3	nd
Condensed tannins ^{††}	131.6	0.4	1.5

nd, not done, [†] Expressed as g tannic acid equivalent per kg DM, ^{††} Expressed as g leuco-cyanidine equivalent per kg DM.

Table 3. Blood parameters and daily weight gain of lambs when fed on each of the three diets

	Diet			SE	P
	Grass	Acacia	Acacia / PEG		
Plasma urea (mmol/l)	4.38 ab	3.77 b	4.91 a	0.33	0.048
Haemoglobin (g/dl)	9.37 b	9.99 a	10.01 a	0.17	0.017
Inorganic phosphates (mmol/l)	1.72	1.84	1.85	0.05	0.148
Daily weight gain (g/day)	110.2 a	57.7 b	110.7 b	10.1	0.0003

Means in the same line with different letters differ ($P < 0.05$).

After the start of the trial during the first grazing period, FECs decreased for the three diets by comparison to the initial FECs measured in all lambs before the start of the experiment. This decrease is probably related to the improvement of the nutritional conditions of the animals leading subsequently to a better immune control of infestation by digestive gastrointestinal helminths. The mean FEC obtained in acacia group is significantly lower than that of lambs on grass-diet (849.1 epg vs 1001.8 epg respectively, $p < 0.05$). This trend indicates an overall reduction of the excretion of gastrointestinal nematodes eggs following the ingestion of acacia foliage. However, this effect might have been underestimated here due to the interference of other factors such as variation of the weight of the faecal bulk in relation to the diet used, and a potential carry-over

and/or partial lethal effect of acacia on the helminths population like in other CT sources such as Quebracho (Paolini *et al.*, 2003b) and Sulla (Niezen *et al.*, 2002). Numerous observations lead to logically admit that condensed tannins (CT) are responsible for the reduced excretion of nematode eggs (Kabasa *et al.*, 2000; Paolini *et al.*, 2003b). Under certain circumstances CT could increase by pass proteins, thus increase the flow of essential amino acids in the intestine of ruminants. This effect could result in enhanced immunity to gastrointestinal nematodes as suggested by Coop and Kyriazakis (2001). However, our results indicate the presence of a lower amount of digestible protein in acacia than in grass diets, since during their grazing period on acacia lambs had the lowest average daily weight gain and plasmatic urea concentrations (57.7 g/day and 3.77 mmol/l, respectively) when compared to the grass-group.

IV – Conclusion

This preliminary study demonstrated an evident anti-nematode effect of acacia as shown by the reduction of FECs. Furthermore, these results obtained under field conditions, might indicate that this anti-nematodes effect of acacia could be potentially exploited, as noted here, under usual management conditions of sheep in the semi-arid part of Tunisia.

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