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Abstract. To test the hypothesis that the beneficial anthelmintic effect of the consumption of moderate amounts of tannins is not always accompanied by anti-nutritional effects, we used 48 Cashmere goats randomly assigned to 2 treatments [supplemented with heather (6.4% total tannins) *vs* non-supplemented]. All goats grazed continuously from May to September, under practical conditions in a mountain area of Northern Spain. Heather percentage in the diet of supplemented animals reached 29.1%. Supplementation with heather reduced the mean number of nematode eggs in faeces ($P < 0.001$) and the mortality rate (at the end of the grazing period, 2 goats had died in the group supplemented with heather *vs* 8 in the group without supplementation; $P < 0.05$). Rumen ammonia concentration was markedly decreased in goats receiving tannin-containing heather (160 *vs* 209 mmol/l; $P < 0.01$), which agrees with the well known effect of tannins on the proteolysis of feed protein. On the contrary, volatile fatty acid (VFA) concentrations were significantly greater in supplemented animals (63.0 *vs* 53.6 mmol total VFA/L) due mainly to acetic and propionic acid increases (approx. 26 and 16%, respectively), in spite of valeric and branched-VFA decreases, which suggests that ruminal fermentation was not adversely affected by tannin consumption. These data, together with a lower loss of live weight ($P < 0.10$) and body condition score ($P < 0.05$) in heather supplemented goats, support the absence of any apparent nutritional cost that counteracted the beneficial anthelmintic effect of the supplementation of grazing goats with tannin-containing heather.

Keywords. Gastrointestinal nematode – Nutrition – Rumen fermentation – Tannin – Heather.

L'effet anthelmintique de la supplémentation par la bruyère n'est pas toujours associé avec les effets anti-nutritionnels chez les caprins en pâturage

Résumé. *Pour évaluer l'hypothèse selon laquelle l'effet bénéfique anthelmintique de la consommation de quantités modérées de tannins n'est pas toujours accompagné d'effets anti-nutritionnels, 48 chèvres Cachemire ont été réparties au hasard en 2 traitements [supplémenté avec de la bruyère (6,4% tannins totaux)* vs *non supplémenté]. Toutes les chèvres ont pâturé sans interruption de mai à septembre, en conditions d'élevage, dans une région de montagne du Nord de l'Espagne. Le pourcentage de bruyère dans le régime des animaux supplémentés a atteint 29,1%. La supplémentation par la bruyère a réduit le nombre moyen d'œufs de nématode dans les fèces ($P < 0,001$) et le taux de mortalité (en fin de pâturage, 2 chèvres étaient mortes dans le groupe supplémenté par la bruyère* vs *8 dans le groupe sans supplémentation ; $P < 0,05$). La concentration ruminale en azote ammoniacal a été nettement diminuée chez les chèvres recevant la bruyère (160* vs *209 mmol/l ; $P < 0,01$), ce qui est conforme à l'effet connu des tannins sur la protéolyse des protéines alimentaires. Par contre, les concentrations d'acides gras volatils (AGV) ont été sensiblement plus élevées chez les animaux supplémentés (63,0* vs *53,6 mmol AGV/L total), en raison surtout d'augmentations des acides acétique et propionique (26 et 16%, respectivement), malgré des diminutions des acides valérique et branchés. Ce résultat suggère que la fermentation ruminale n'a pas été compromise par la consommation de tannins. Ces résultats, ainsi qu'une moindre perte de poids vif ($P < 0,10$) et d'état corporel ($P < 0,05$) chez les chèvres supplémentées,*

montrent qu'il n'y a eu aucun coût alimentaire apparent ayant contrecarré l'effet bénéfique anthelmintique de la supplémentation des chèvres avec la bruyère riches en tannins.

Mots-clés. *Nématode gastro-intestinal – Nutrition – Fermentation ruminale – Tannin – Bruyère.*

I – Introduction

An increasing number of studies relates the consumption of tannin-rich plants by parasitized ruminants to a regulation of nematode populations, thereby reducing the dependence on conventional chemotherapy and favouring a sustainable control of gastrointestinal nematode parasitism (Hoste *et al.*, 2006). However, it is widely accepted that the consumption of tannin-containing plants by parasitized animals may result in either favourable or detrimental net effects depending on whether or not the beneficial anthelmintic effects overweigh its nutritional cost on the host (Houdijk and Athanasiadou, 2003). Tannins, on the other hand, have also been reported to exert potentially beneficial effects, associated basically with reduced protein losses from the rumen, when consumed in moderate concentrations (Waghorn, 1996; Barry and McNabb, 1999).

Based on our earlier work with grazing goats supplemented with tannin-containing heather (Osoro *et al.*, 2007a,b), we hypothesized that the beneficial anthelmintic effect of the consumption of moderate amounts of tannins may not always be accompanied by anti-nutritional effects. The following study was conducted to test this hypothesis.

II – Materials and methods

The experiment was conducted in a mountain area of the northwest of Spain (Sierra de San Isidro, Illano, Asturias) dominated by shrubby heather-gorse vegetation. Several plots of pasture were created in 2001 by removing the shrubs and dressing and sowing perennial ryegrass (*Lolium perenne* L.) and white clover (*Trifolium repens* L.).

Forty-eight lactating Cashmere goats (36.8 ± 1.13 kg at the beginning of the trial), balanced for live weight (LW) and body condition score (BCS), were randomly assigned, together with their single kids, to 2 treatments: supplemented with heather *vs* non-supplemented. Each of the two groups was confined to an area of approx. 10,000 m^2 for the whole grazing season (from May to September). Supplemented goats were offered, in the morning and every 3 days, freshly cut heather *ad libitum*, whereas the others received no supplement. All animals were reared outdoors under farm conditions (i.e., not excluding nematode infections). The heather offered was mostly composed of *Calluna vulgaris* (L.) Hull (61%), with 25% *Erica umbellata* L., 12% *E. cinerea* L., 1% *E. tetralix* L. and 1% *Daboecia cantabrica* (Hudson) C. Koch.

In August, following an overnight fast, ruminal fluid was collected from each goat by stomach tube, and strained through 2 layers of gauze. Immediately afterwards, 4 ml were acidified (4 ml 0.2 N HCl) for ammonia determination and 0.8 ml were deproteinized (0.5 ml of 2% metaphosphoric and 0.4% crotonic acids, wt/vol, in 0.5 N HCl) for volatile fatty acids (VFA) determination. All samples were stored at -30°C until analysis. Ammonia concentration was determined by a modified colorimetric method (Weatherburn, 1967) and VFA by gas chromatography, using crotonic acid as internal standard (Ottenstein and Bartley, 1971), both in centrifuged samples.

Spot samples of goat faeces were individually collected from each animal by rectal grab sampling approx. once per month, from May to September, to assess gastrointestinal nematode egg excretion. The numbers of gastrointestinal nematode eggs/g of faeces were estimated using the modified McMaster technique (MAFF, 1978) with sodium chloride as the flotation medium.

Pasture samples collected in August were analyzed for ash and N (AOAC, 1999). Neutral and acid

detergent fibre (NDF and ADF) and acid detergent lignin (ADL) were determined by the methods of Goering and van Soest (1970) and van Soest *et al.* (1991). Samples of green shoots (i.e., those parts expected to be consumed by goats) of the heather offered to the animals were collected at the same time for chemical composition (ash, N, NDF, ADF and ADL) and total tannins (TT). Assays for TT were conducted following the Folin-Ciocalteu technique in combination with polyvinyl-polypyrrolidone, using tannic acid (Merck) as the reference standard (Makkar *et al*., 1993).

The percentage of heather incorporated in the diet of each supplemented goat was also estimated in August using the n-alkane method (Mayes *et al*., 1986) modified by Ferreira *et al*. (2005). All animals were weighed and their BCS was assessed on a scale of 1 to 5 (Russel, 1990), at the beginning and the end of the experiment and also at monthly intervals.

Data on faecal egg counts (FEC), LW and BCS were analyzed by repeated measures analysis (Littell *et al*., 1998), using the MIXED procedure of the Statistical Analysis System program (SAS, 1999). The first measurement in May was used as the covariate and the animals were nested within the treatment and used as the error term to contrast the effect of heather supplementation. Data on FEC were log transformed ($\log_{10}$ x + 1) to normalize their distribution. Rumen parameters were analyzed as a one-way analysis of variance, using the GLM procedure, and mortality rate by chi-squared test (SAS, 1999).

III – Results and discussion

Heather percentage in the diet of supplemented animals showed a mean value of 29.1% (±6.80), which was very close to that observed in a previous experiment conducted under similar conditions (Osoro *et al*., 2007b). Chemical composition of the pasture and the offered heather in August is shown in Table 1. Given the low content of protein in these shrubs, it seems very unlike that the anthelmintic effect of heather supplementation may be due to a higher availability of protein (Coop and Kyriazakis, 2001), although there might be a greater availability of non-degradable dietary protein as a consequence of the intake of tannins (Barry and McNabb, 1999; Frutos *et al*., 2000).

Table 1. Chemical composition of pasture and heather (g/kg DM, except for DM that is g/kg)

	DM	OM	CP	NDF	ADF	ADL	TT†
Pasture	328	949	143	590	269	25	nd
Heather	423	979	61	557	463	224	64

†g tannic acid equivalents/kg DM; nd = not determined.

In agreement with results observed in previous years (Osoro *et al*., 2007a,b), supplementation with heather reduced the mean number of nematode eggs in goat faeces ($P < 0.001$; Fig. 1). It is probably noteworthy that 7 of the non-supplemented goats (those with the highest FEC in the two samplings performed in August) died before the September sampling, which explains the decrease in FEC observed in the figure. In this respect, supplementation with heather significantly reduced the mortality rate ($P < 0.05$): at the end of the grazing period, 2 goats receiving heather had died *vs* 8 in the group receiving no supplement. Other comparative results on goats receiving or not sainfoin hay (Paolini *et al*., 2005) support this result on a better resilience (lower death rate) when goats receive tanniniferous supplementation.

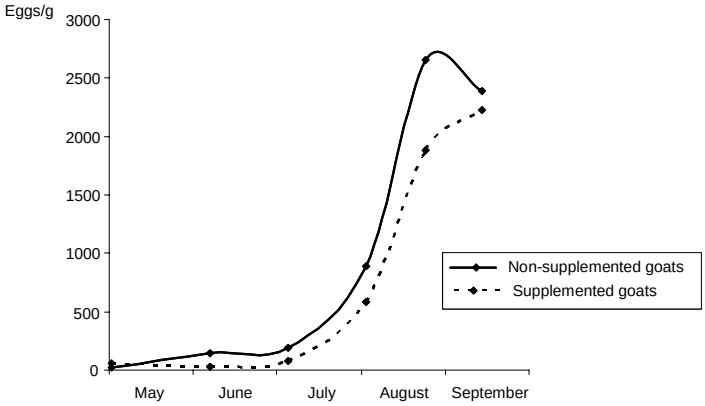

Fig. 1. Backtransformed values of FEC (eggs/g) during the whole experimental season in grazing goats supplemented or not with heather (mean log transformed values 2.20 *vs* 2.66 for supplemented *vs* non-supplemented animals; sed = 0.115).

Table 2 shows ammonia and volatile fatty acid concentrations in the ruminal fluid of experimental animals. Rumen ammonia concentration was markedly decreased in goats receiving tannin-containing heather ($P < 0.01$), which agrees with the well known effect of tannins on the proteolysis of feed protein (Waghorn, 1996). On the contrary, VFA concentrations were significantly greater in supplemented animals ($P < 0.05$), which suggests that ruminal fermentation was not adversely affected by tannin consumption. This higher concentration of VFA was mainly due to an increase of the acetic acid (approx. 26%), but also of the propionic acid (approx. 16%). On the other hand, valerate and branched-VFA (referred as "Others" in Table 2) decreased, which is in line with the effect of tannins on proteolysis (Waghorn, 1996). Molar proportions of VFA were also modified by the supplementation with heather ($P < 0.001$), with increases of acetate and decreases of butyrate and others, indicating a divergence in the fermentation pathways. There may be two possible explanations for the higher VFA concentration in goats consuming heather. On the one hand, heather incorporation in the diet did not reduce extensively the intake of pasture, increasing total feed intake and therefore VFA production. On the other hand, pasture intake was partially substituted by heather, but this improved the efficiency of ruminal fermentation of the diet.

Table 2. Effects of heather supplementation on ammonia (mg/l) and VFA (mmol/l) concentrations in the ruminal fluid of grazing goats

	Heather supplementation				P=
	NO	sem	YES	sem	
Ammonia	209.40	11.250	159.70	10.110	0.0021
Total VFA	53.61	2.286	62.98	2.619	0.0119
Acetate	36.11	1.937	45.47	1.980	0.0018
Propionate	8.80	0.419	10.25	0.596	0.0630
Butyrate	5.48	0.288	5.08	0.287	0.3356
Others†	3.22	0.201	2.17	0.092	0.0001

†Calculated as the sum of valerate, isovalerate, isobutyrate and caproate.

Heather supplementation was also responsible for the tendency to a lower loss of live weight (-1.25 *vs* -1.77 kg; $P < 0.10$) and body condition score (-0.12 *vs* -0.25; $P < 0.05$) throughout the experiment. Negative live weight gains in parasitized animals, as observed in previous years

(Osoro *et al*., 2007a,b) was likely a consequence of limited availability and quality of pasture, and increase of parasite burden during the grazing season (Coop and Kyriazakis, 2001). Notwithstanding, these losses were mitigated by the effect of heather intake.

IV – Conclusion

The results of this study did not show any apparent nutritional cost that counteracted the anthelmintic effect of the supplementation of grazing goats with heather. Therefore, they support the hypothesis that the beneficial anthelmintic effect of the consumption of moderate amounts of tannins may not always be accompanied by substantial anti-nutritional consequences. Further research, including estimation of pasture and heather intake, would contribute to explain the effects of heather supplementation on nutrition and performance of grazing goats. Eventually, it would also contribute to the development of a sustainable method to control gastrointestinal nematode parasitism in extensive goat production systems.

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