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Effects of the use of heather as anthelmintic in goats infected with *Trichostrongylus colubriformis* on ruminal fermentation and digestibility

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Abstract. Previous field studies have supported the absence of a nutritional cost outweighing the beneficial anthelmintic effect of supplementing the diet of grazing goats with tannin-containing heather. In order to further research in this regard, an experiment was conducted indoors with 18 does artificially infected with *Trichostrongylus colubriformis*. The goats were offered lucerne hay for 6 weeks and then assigned to 3 treatments (diets): lucerne hay (L), 70% lucerne hay + 30% heather containing 64 g of tannic acid equivalents/kg DM (LH), and LH + polyethylene glycol (35 g PEG/animal and day; LH+PEG). Rumen fluid was obtained from each animal after 10 (period 1) and 36 (period 2) days, and afterwards total faecal output was collected for 5 days to assess gastrointestinal nematode egg excretion, and apparent digestibilities of DM and CP. Total daily faecal egg excretion was reduced in does consuming heather (491,216 for L *vs* 234,311 and 194,356 for LH+PEG and LH; P<0.05). Volatile fatty acid concentrations were greater in those animals (115 *vs* 102 *vs* 84 for LH, LH+PEG and L, respectively, P<0.05) but the use of PEG increased DM and CP digestibilities (P<0.05). *In vitro* gas production results suggest an adaptation of the rumen microbiota in goats supplemented with heather that was not reflected in differences between digestibility coefficients in periods 1 and 2. The fact that LH+PEG significantly improved the apparent digestibilities of DM (6%) and CP (13%) when compared to LH, but with both reducing egg excretion in the same proportion, might suggest that the threshold of tannins requested to obtain anthelmintic effects is probably quite low.

Keywords. Goat – Heather - Nutrition – Parasite – Rumen – Tannin.

Effets de la bruyère distribuée à des chèvres infestées par* Trichostrongylus colubriformis *sur la fermentation ruminale, la digestibilité et la dynamique d'infestation

Résumé. *Des études précédentes ont montré l'absence d'un coût nutritionnel contrebalançant l'effet anthelminthique favorable de la supplémentation de chèvres au pâturage avec de la bruyère contenant des tannins. Afin d'examiner en détail cette question, une expérience a été menée sur 18 chèvres adultes artificiellement infestées par* Trichostrongylus colubriformis*. Les chèvres ont reçu du foin de luzerne pendant 6 semaines, puis elles ont été réparties en 3 traitements (régimes) : foin de luzerne (L), 70% luzerne + 30% bruyère contenant 6,4 g d'équivalents d'acide tannique/kg MS (LH), et 70% luzerne + 30% bruyère + polyéthylène glycol (35 g PEG/animal et jour; lot LH+PEG). Du liquide ruminal a été collecté de chaque animal après 10 jours (période 1) et 36 jours (période 2) puis, la production fécale totale a été recueillie pendant 5 jours pour évaluer l'excrétion d'œufs de nématodes gastro-intestinaux et les digestibilités apparentes de MS et MP. L'excrétion fécale totale d'œufs a été réduite chez les chèvres recevant de la bruyère contenant des tannins (491,216 pour le lot L contre 234 311 et 194 356 pour les lots LH+PEG et LH; P<0,05). Les concentrations d'acides gras volatils ont été plus élevées chez les animaux recevant de la bruyère (115 vs 102 vs 84 pour LH, LH+PEG et L, respectivement, P<0,05) mais l'utilisation de PEG a augmenté les digestibilités de MS et PB (P<0,05). Les résultats de production ruminale de gaz in vitro suggèrent une adaptation de la microflore du rumen chez les chèvres supplémentées avec de la bruyère qui n'a pas été reflétée dans les différences entre coefficients de digestibilité des périodes 1 et 2. Le fait que dans le groupe LH+PEG, les digestibilités de MS (6%) et de MP*

PC (13 %), soient améliorées de façon significative et que dans les deux lots, LH et LH + PEG, la consommation de bruyère rédui les excrétions d'œufs dans la même proportion, suggèrent que le seuil de tanins requis pour obtenir les effets anthelminthiques est probablement assez bas.

Mots-clés. *Bruyère – Chèvre – Nutrition – Parasite – Rumen – Tannins.*

I – Introduction

It is nowadays widely accepted that parasite-infected goats that consume tannin-containing plants may show improved resistance and resilience to parasites, and significant decreases in faecal egg counts and gastrointestinal nematode burden (Coop and Kyriazakis, 2001; Osoro *et al.*, 2007). However, it is also widely thought that the consumption of tannins may result in a detrimental net effect if their anthelmintic action is outweighed by an associated nutritional cost to the host (Houdijk and Athanasiadou, 2003).

Previous field studies performed by our group (Osoro *et al.*, 2007; Frutos *et al.*, 2008) supported the absence of any substantial nutritional cost counteracting the beneficial anthelmintic effect of supplementing the diet of grazing goats with tannin-containing heather (*Ericaceae* spp.). In order to further research in this regard, an experimental trial was carried out indoors with does artificially infected with *Trichostrongylus colubriformis*.

II – Materials and methods

Two weeks before the experiment started, all goats were orally treated with ivermectin (Oramec, Merial, Lyon, France).

Eighteen adult Cashmere does (approx. 5 years old) were experimentally infected with 6,000 L3 of *T. colubriformis*. All the animals were offered lucerne hay *ad libitum* for 6 weeks and then housed individually and assigned to three treatments (diets): lucerne hay (L), 70% lucerne hay + 30% heather (LH), and 70% lucerne hay + 30% heather + polyethylene glycol (LH+PEG). Diets (30 g DM/kg $LW^{0.75}$) were offered twice a day. The heather was cut every three days and frozen until offered to the animals. Goats on treatment LH+PEG were orally drenched with 70 ml of a water solution containing 35 g PEG 6000 (Fluka Chemie GmbH, Buchs, Switzerland) every day immediately before the morning meal. Clean water was always available.

After 10 (period 1) and 36 (period 2) days on treatments (which would correspond, respectively, to 52 and 78 days post-infection), ruminal fluid was collected from each goat by stomach tube, and strained through 2 layers of gauze. The pH was measured and then 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 -30ºC until analysis. Ammonia concentration was determined by a colorimetric method (Weatherburn, 1967) and VFA by gas chromatography, using crotonic acid as the internal standard (Ottenstein and Bartley, 1971), both in centrifuged samples.

Afterwards, total faecal output was collected for 5 consecutive days (days 12-16 and 38-42) to assess gastrointestinal nematode egg excretion and apparent digestibilities of dry matter (DM) and crude protein (CP). Faeces were collected daily from each animal, weighed, thoroughly mixed and subsampled. Aliquots for digestibility (10% of faeces collected per day) from each goat were bulked, and the pooled sample dried to constant weight, ground and analysed for DM and CP.

A further aliquot per animal and day was used to assess total gastrointestinal nematode egg excretion, using the modified McMaster technique (MAFF, 1978) with sodium chloride as the flotation medium, in which every egg is regarded as equivalent to 15 eggs per g fresh faeces.

The *in vitro* ruminal fermentation of the heather offered was studied using a modification of the gas production technique described by Theodorou *et al.* (1994). At the beginning of period 2, ruminal fluid was not only collected for ammonia and VFA analysis (as previously described) but also to composite three inocula (one per treatment). Eighteen samples of heather (3 inocula x 2 replicates x 3 flasks/replicate), ground through a 1-mm screen (≈ 500 mg), were incubated in 125 ml serum flasks at 39ºC with 10 ml strained rumen fluid and 40 ml phosphate-bicarbonate medium. Gas production was determined by measuring head-space gas pressure at 2, 4, 6, 8, 10, 13, 17, 22, 28, 35, 48, 72, 96 and 118 h post-inoculation. Pressure values, corrected for the quantity of substrate OM incubated and gas released from blanks (*i.e.*, rumen fluid plus buffer medium, without substrate; 6 in total), were used to generate gas volume estimates using a predictive equation derived from earlier simultaneous pressure and volume measurements (Hervás *et al.*, 2005).

Lucerne hay and heather samples were analysed for DM (ISO 6496:1999), organic matter (OM, ISO 5984:2002) and CP (ISO 5983-2:2005). Neutral and acid detergent fibre (NDF and ADF) and acid detergent lignin (ADL) were determined by the method of Goering and Van Soest (1970) and Van Soest *et al.* (1991), using an Ankom^{2000} Fiber Analyzer (Ankom Technology Corp., Macedon, NY, USA). Assays for heather total phenolics (TP) and tannins (TT) were conducted following the Folin-Ciocalteu technique in combination with polyvinyl-polypyrrolidone, using tannic acid (Merck, Damstadt, Germany) as the reference standard (Makkar *et al.*, 1993).

In vivo ruminal characteristics, digestibility coefficients and total gastrointestinal nematode egg excretion data were analysed by repeated measures analysis, using the MIXED procedure of the SAS 9.1 (SAS Institute Inc., Cary, USA).

III – Results and discussion

The chemical composition of the lucerne hay and heather is shown in Table 1. Given the low content of protein in the heather, the anthelmintic effect attributed to the presence of tannins in these shrubs might have been counteracted by the lower availability of protein in treatments LH and LH+PEG (Coop and Kyriazakis, 2001). Nonetheless, total daily faecal egg excretion was strongly reduced in does consuming heather ($P<0.05$; Fig. 1).

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

	DM	OM	CP	NDF	ADF	ADL	TP†	TT†
Lucerne hay	897±0.9	893±5.3	160±2.5	420±18.0	317±13.3	66±4.8	nd	nd
Heather	469±1.4	977±0.3	63±2.7	505±10.4	378±10.2	265±44.2	96±7.2	64±6.2

† g tannic acid equivalents/kg DM; TP= total phenolics; TT= total tannins; nd = not determined.

Differences between treatments were stronger in the second period due to the noticeable increase in the number of eggs excreted by animals receiving no tannin-containing heather. On the other hand, no significant differences were observed between treatments LH and LH+PEG, which might indicate that inhibition of tannins by PEG was not completed, as previously suggested by Frutos *et al.* (2004), and the amount of free tannins was enough to exert an anthelmintic action. In addition, it cannot be categorically ruled out that other plant secondary metabolites present in the heather might have also contributed to the nematode control, which would require further research.

Surprisingly, ammonia concentration in the ruminal fluid of the experimental animals showed no significant differences between treatments (on average 190 ± 6.2 mg/l; $P>0.10$), when a reduc-

tion in goats receiving tannins had been expected on the basis of most published results. Volatile fatty acid concentrations, however, as previously observed (Osoro *et al.*, 2007; Frutos *et al.*, 2008), were greater in animals consuming tannins (Fig. 2), with mean values of 115 *vs* 102 *vs* 84 for LH, LH+PEG and L, respectively (P<0.05). The addition of PEG to the diet did not significantly modify the VFA concentrations (LH *vs* LH+PEG; P>0.10).

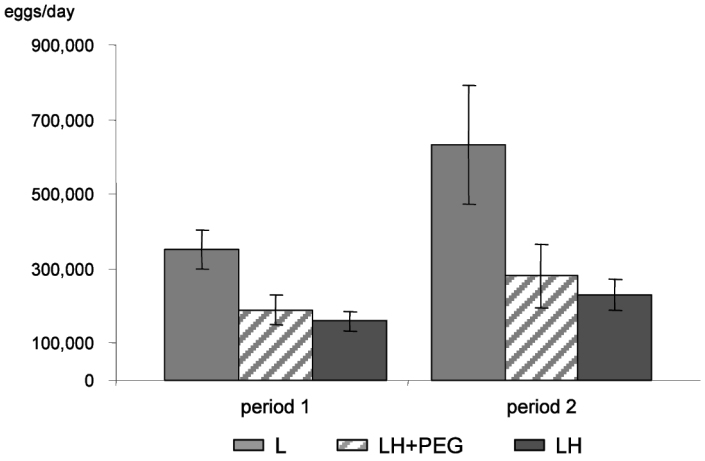

Fig. 1. Total daily faecal egg excretion in goats experimentally infected with *T. colubriformis,* fed lucerne hay (L), 70% lucerne + 30% heather (LH), and LH + polyethylene glycol (LH+PEG). ‡ = s.e.

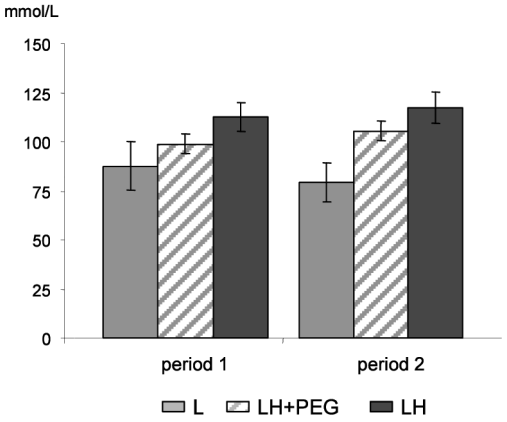

Fig. 2. Ruminal concentration of total VFA in goats fed lucerne hay (L), 70% lucerne hay + 30% heather (LH), and LH + polyethylene glycol (LH+PEG). ‡ = s.e.

However, when LH and LH+PEG were compared, the consumption of the tannin-binding agent increased DM (6%) and CP (13%) apparent digestibility coefficients (P<0.05; Fig. 3). No significant differences were observed either for period or for the interaction period x treatment which dissuades from considering adaptation.

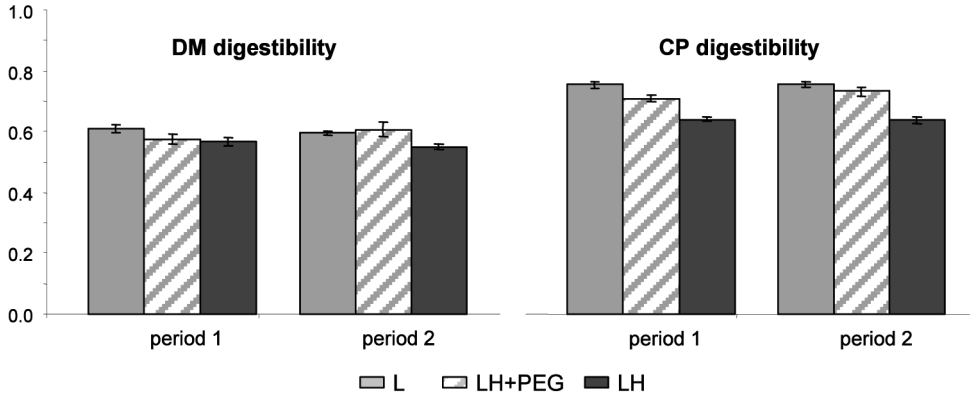

Fig. 3. Apparent digestibilities of DM and CP in goats fed lucerne hay (L), 70% lucerne + 30% heather (LH), and LH + polyethylene glycol (LH+PEG). ‡ = s.e.

Nevertheless, results from the *in vitro* ruminal fermentation study showed a higher rate and extent of gas production when the heather was incubated with rumen inoculum derived from goats fed these shrubs, suggesting an adaptation of the rumen microbiota to tannin consumption (see Fig. 4). That adaptation, however, was insufficient to compensate for the depressive effect of these phenolics on ruminal fermentation, for the best results were observed for incubations with rumen fluid from goats drenched with PEG.

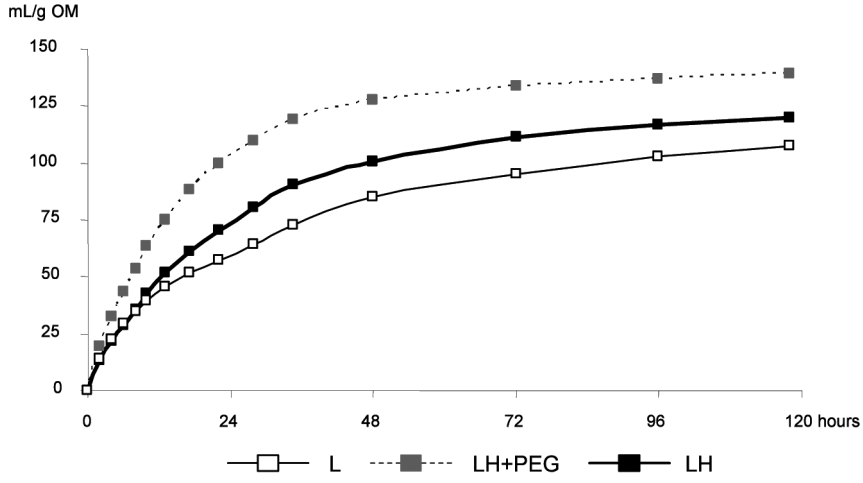

Fig. 4. *In vitro* cumulative gas production profiles of heather incubated with rumen inoculum from goats fed lucerne hay (L), 70% lucerne hay + 30% heather (LH), and LH + polyethylene glycol (LH+PEG).

IV – Conclusion

Although this study shows the existence of some mild detrimental effects of heather tannins on ruminal fermentation and diet digestibility, they are not as negative as those observed by other authors using other tannin-containing plant species (*e.g.*, Silanikove *et al.*, 1994; Tiemann *et al.*, 2008). In fact, some positive effects, such as increased VFA concentration, were found.

In addition, it is nowadays widely admitted that the concentration of tannins in the diet is a key factor modulating the balance between their potential positive and negative effects. In this trial, the fact that the inclusion of PEG (LH+PEG) reduced gastrointestinal nematode egg excretion in the same proportion as the LH treatment suggests that the threshold of tannins needed for anthelmintic effects is probably quite low and that the proportion of heather included in the diet to this end could be lowered. Since the question of the lowest threshold of tannins required to obtain anthelmintic effects has hardly been addressed, further research on this issue would be highly recommendable.

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