

Research Article

Cite this article: Bordes L *et al* (2024). Pellets enriched with healthy hay and quebracho are not sufficient to control gastrointestinal nematodes in meat sheep commercial flocks. *Parasitology* **151**, 1380–1385. <https://doi.org/10.1017/S0031182024001409>

Received: 1 July 2024
Revised: 16 October 2024
Accepted: 16 October 2024
First published online: 15 November 2024


Keywords:

condensed tannins; gastro-intestinal nematodes; sheep

Corresponding author:

Léa Bordes;
Email: lea.bordes@envt.fr

Pellets enriched with healthy hay and quebracho are not sufficient to control gastrointestinal nematodes in meat sheep commercial flocks

Léa Bordes¹ , Corentin Souchon², Alice Claessens², Sophie Lavigne³, Geneviève Bouix², Margaux Goyenette³, Laurence Sagot³, Christelle Grisez¹, Guy-Gérard Merlande² and Philippe Jacquet¹

¹IHAP, UMR 1225 INRAE/ENVT, Université de Toulouse, Toulouse, France; ²GEODE, Coopérative ovine, Roussines, France and ³Centre Interrégional d'Information et de Recherche en Production Ovine (CIIRPO), Saint-Priest-Ligoure, France

Abstract

The emergence of AH multiresistant GIN compromises sustainability of grassland sheep farming worldwide. Plants rich in condensed tannins are an alternative method of parasitism management that is currently being explored. Feed supplementation trials with pellets rich in sainfoin (*Onobrychis viciifolia*) and quebracho (*Schinopsis* spp.) were carried out. Three meat sheep farms in western France took part in the study and a total of 4 trials were carried out.

During these 21-day trials, the ewes were returned to the sheepfold and half of them received a balanced ration supplemented with 70 g day⁻¹ of healthy hay and quebracho pellets, while the other half received the same ration supplemented with 70 g day⁻¹ of lucerne pellets. Fecal egg counts (FEC) were carried out at the start and end of each trial, and nematode species were identified by real-time PCR after larval culture. At D0, FEC were similar in both groups for all 4 trials. Proportions of species infecting the ewes varied from 1 trial to another: *Haemonchus contortus* was predominant in summer and *Trichostrongylus colubriformis* in winter. At D21, there were no significant differences in FEC between groups. Helminthofauna were not significantly different between groups, except for 1 trial where the proportion of *H. contortus* was reduced in the group supplemented with condensed-tannin pellets. The use of condensed tannins still requires additional studies to be advised as an effective method to manage gastrointestinal nematodes in farm.

Introduction

In southwestern France, the use of pasture for most of the year is the rule in dairy and meat sheep systems. This has many advantages in terms of food availability, costs saving for farmers and animal welfare. However, the main concern with natural grazing is the gastrointestinal nematodes (GIN) infection of animals by. These parasites lead to losses in milk, meat and wool production, sometimes mortality and also impact adult reproductive performances (Mavrot *et al.*, 2015).

In France, 3 GIN species are considered to be dominant according to their pathogenesis and relative abundances: *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. In order to control these GIN species, farmers usually have recourse to chemical anthelmintic treatments which are easy-to-use and inexpensive and are supposed to be effective. However, repeated and systematic treatments during decades have led to the emergence of anthelmintic resistance (Falzon *et al.*, 2014). The first family of anthelmintic molecules which was impacted by resistance was the benzimidazoles (BDZ). Widely used since the 1960s, the resistance of GIN to this family is now widely spread in Europe (Vernerova *et al.*, 2009; Papadopoulos *et al.*, 2012) and in France (Chartier *et al.*, 1998; Geurden *et al.*, 2014; Krücken *et al.*, 2024). To overcome this situation, a switch to macrocyclic lactones (ML) family had been made in both dairy and meat sheep farms. Nevertheless, this change of family was not accompanied by a change in treatment's practices and resistance to ML appeared very quickly (Borgsteede *et al.*, 2007; Papadopoulos, 2008; Scheuerle *et al.*, 2009). Thus, recently, the first cases of multiresistance of GIN to several families of anthelmintics were reported in Europe (Sargison *et al.*, 2007; Traversa *et al.*, 2007; Taylor *et al.*, 2009; Voigt *et al.*, 2012; Van den Brom *et al.*, 2013; Krücken *et al.*, 2024) including France (Paraud *et al.*, 2016; Milhes *et al.*, 2017; Cazajous *et al.*, 2018; Bordes *et al.*, 2020; Jouffroy *et al.*, 2023).

To face the spread of anthelmintic resistance in GIN, alternative or complementary solutions have been studied and are based on 3 main actions: targeting the parasites in the host (targeting selective treatment, bioactive plants...) (Ahuir-Baraja *et al.*, 2021), increasing the host's resistance (protein supplementation, genetic selection of host resistance...) (Aguerre *et al.*, 2018; Thorne *et al.*, 2024) and improving the pasture's management (cell grazing, pasture rotation...) (Ruiz-Huidobro *et al.*, 2019). One of the alternatives to chemical anthelmintic

treatments is the use of bioactive plants, showing anthelmintic effects. This is a short-term solution as bioactive plants are acting directly on the parasites. Their anthelmintic effect is mainly based on their ability to produce tannins, which are secondary metabolites, divided in 2 main groups : hydrolysable tannins and condensed tannins (CTs), the latter only being involved in the control of GIN (Hoste *et al.*, 2006). CTs are widespread in the plant kingdom as they are found in both angiosperms and gymnosperms (Stafford and Lester, 1980). The highest levels of CTs are found in legumes such as healthy hay (*Onobrychis viciifolia*), bunched hedizarum (*Sulla coronaria*), birdsfoot trefoil and big trefoil (*Lotus corniculatus* L. and *Lotus pedunculatus*) and Sericea lespedeza (*Lespedeza cuneata*) (Mueller-Harvey *et al.*, 2019). Other forbs such as chicory (*Cichorium intybus*) and plantain (*Plantago lanceolata*) may also have interesting tannin contents. Finally, some chestnut barks, such as quebracho (*Schinopsis* spp.), have high CTs content (Athanasiadou *et al.*, 2001), which can supplement or increase tannin intake in animals using barks extracts in diet.

Several studies showed the effect of CTs in GIN *in vitro* conditions: inhibition of the exsheathment of L3 larvae (Brunet *et al.*, 2007) associated with disruptions between cuticle and sheath (Brunet *et al.*, 2011) and decreased larval motility (Paolini *et al.*, 2004; Manolaraki *et al.*, 2010). *In vivo* studies on sheep and goats have also been carried out to understand the mechanisms of action of tannins: exposure of worms to tannins result in a decrease of motility in adult worms (Paolini *et al.*, 2004), a reduction of the number of eggs in the uterus of adult female worms (Paolini *et al.*, 2003a; Heckendorn *et al.*, 2006, 2007; Manolaraki *et al.*, 2010; Gaudin *et al.*, 2016) and a decrease of fecal egg excretion (Paolini *et al.*, 2003a, 2003b; Manolaraki *et al.*, 2010). These effects were observed on susceptible as well as multiresistant GIN populations (Gaudin *et al.*, 2016). When CTs and infective larvae were given simultaneously in experimental studies, the number of adult worms established in the digestive system was decreased for *T. colubriformis* and *H. contortus* compared to control groups (Paolini *et al.*, 2003b; Heckendorn *et al.*, 2006). Initially, CTs extracts or hay were used in these previous studies. In order to improve the conservation and stability of CTs and facilitate the distribution to animals and the storage in farms, pellets were developed with healthy hay (Terrill *et al.*, 2007; Gaudin *et al.*, 2016) and *Sericea lespedeza* (Gujja *et al.*, 2013; Kommuru *et al.*, 2014, 2015) with expected anthelmintic effects.

According to these encouraging experimental results, it is now essential to test CTs effect on field conditions with a simple protocol for sheep breeders. Three commercial farms from the western France, a region undergoing strong GIN pressure and multi-resistant nematodes were selected in this study. We evaluated the impact of a diet complementation based on commercial pellets enriched with healthy hay and quebracho on the intensity of GIN egg excretions and on the composition of GIN communities in animals naturally infected.

Materials and methods

Experimental design

In order to propose practical solutions to sheep breeders, we chose commercially available tannin-rich plant pellets (Nativiamix®, Mg2Mix), to be tested in this study. These pellets have the interest to be more concentrated in CTs than their counterparts composed of healthy hay only, thanks to the presence of quebracho extracts in the pellets with 30% of CTs.

A total of 4 trials were performed on ewes from 3 different farms during the year 2021. Farm 1 and Farm 2 were two commercial sheep farms of the Rouge de l'Ouest (RdO) meat sheep breed and Farm 3 was a flock of the Brebis Vendéenne (V) meat sheep breed. Farm 3 belongs to a research center for sheep breeding, the 'Centre InterRegional d'Information et de Recherche en Production Ovine' (CIIRPO). All farms are located in Western France, in Deux-Sèvres, Vienne and Haute-Vienne French 'départements'. In farms 1 and 2, the ewes used for the study were 2.5 years old and from 2 to 5 years old on the farm 3. In farm 1, 50 ewes were recruited for trials, 51 in farms 2 and 58 in farms 3. Ewes were allowed to graze during 7 to 8 months per year and were maintained indoors twice a year: once in the summer time (2 months) when the grass quantity was no longer sufficient to feed them and once in the winter (2 to 3 months), during lambing period. In the sheepfold, they had a balanced diet covering their nutritional needs. For trial A, B and D the ewes were neither in gestation nor lactating. In trial C, the ewes were at the end of gestation and the beginning to the lambing period.

The trials were performed at the beginning of these indoors periods, on naturally infected ewes. The ewes did not receive any anthelmintic treatment at least 2 months preceding the trials. The numbers of ewes and their description for each trial are presented in Table 1. In each trial, ewes were separated into 2 groups: 1 group received the tannin-rich pellets (Tannin Group) and the other group received lucerne pellets (Control Group). Pellets were distributed daily during 21 days, on a base of 70 g day ewe⁻¹. The daily amount of distributed pellets corresponded to the manufacture's recommendations but the duration of distribution (21 days) was longer than those recommended (10 days only), according with previous successful studies in small ruminants (Paolini *et al.*, 2005; Heckendorn *et al.*, 2006). The diets were equivalent in both groups, and provided the same nutritional intake (protein and energy) to the ewes.

At the beginning of each trial (D0), the ewes received their first ration with pellets and individual fecal samples were collected.

Fecal egg counts and molecular identification of GIN species

Individual fecal egg counts (FEC) were performed by a modified McMaster technique, using a saturated sodium chloride solution of density 1.2 (Raynaud *et al.*, 1970). This technique has a sensitivity of 15 eggs per gram (EPG). The remaining feces were pooled

Table 1. Description of the experimental design

Table 1	Farm 1		Farm 2		Farm 3	
	Trial A		Trial B		Trial D	
Period of cure	Summer 2021		Winter 2021		Summer 2021	
Age of ewes during cure (years)	2.5		2		2.5	
Total number of ewes	50		51		37	
Group	Control	Tannin	Control	Tannin	Control	Tannin
Number of ewes per group	22	28	23	28	19	18
					29	29

Control group, ewes receiving lucerne supplementation; Tannin group, ewes receiving tannin-rich pellets supplementation.

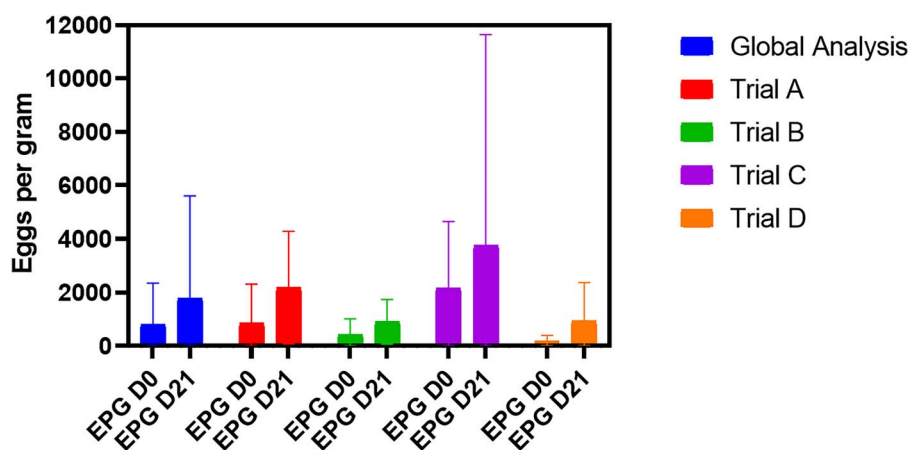


Figure 1. Intensities of egg excretions (means and standard deviations; pooled data from control and tannins groups) in the different trials and for the whole study.

by trial and by group, to obtain L3 larvae in composite larval cultures. All composite larval cultures were incubated in a beaker for 12 days at $24 \pm 1^\circ\text{C}$ and humidified every 2 days with tap water. Third stage larvae (L3s) were recovered by filling the beaker with tap water at room temperature ($\pm 25^\circ\text{C}$) and inverting it on a Petri dish (MAAF, 1986) filled with tap water. L3 larvae have migrated into the water contained in the petri dish and were collected every 12 h, in 3 passes in a same volume of 40–45 mL. Overnight decanting at 4°C in fridge concentrated L3 larvae at the bottom of the tube. The supernatant was discarded, leaving only a 5 mL volume of larval suspension. These suspensions were stored at 4°C until identification step.

Identifications of larvae were done by real-time PCR at the beginning and at the end of each 21 days' trial. Briefly, composite larval cultures were incubated for 12 days at $24 \pm 1^\circ\text{C}$ in jars and humidified every 2 days with tap water. After incubation, the jars were filled with tap water and inverted onto a square Petri dish, also filled with tap water and left for 2 days at room temperature ($\pm 24^\circ\text{C}$). The L3 larvae were recovered every 12 h by collecting the water in the Petri dish. They were concentrated in a 40 to 50 mL Falcon tube and placed in a refrigerator ($\pm 4^\circ\text{C}$) overnight to concentrate the larvae at the bottom of the tube. The supernatant was removed to obtain a larval suspension in a final volume of 5 mL, stored at 4°C until analysis. GIN species molecular identification was performed by real-time PCR (Milhes et al., 2017). This technique specifically detects and quantifies larvae of *H. contortus*, *T. circumcincta* and *T. colubriformis*.

Data and statistical analysis

The distribution of the data and their descriptive analysis were obtained using R software, version 4.2.1 (2022-06-23) (R core Team, 2023) and R Studio software version 2022-07-01 (Posit Team, 2022). Graphs were made with GraphPad Prism version 8.0.1 for Windows (GraphPad Software, 2023).

Different variables were analysed on data collected at D0 and D21:

- EPG at D0 and D21, as a continuous quantitative variable, showed an asymmetrical distribution
- Supplementation, qualitative variable has been divided into 2 categories: 'Control' for the group with lucerne supplementation and 'Tannin' for the group with the tannin-rich pellets supplementation

Several levels of analysis were carried out to assess the anthelmintic effect of pellets rich in CTs on FEC at D0 and D21. Firstly, an overall analysis was carried out, bringing together the results from all the trials. Each condition tested had a number greater than 30

individuals. After an evaluation of the homoscedasticity of the variances using a Fligner test, a Student test was carried out in the case of equal variances and a Welch test in the case of unequal variances. Secondly, statistical analyses for each trial were carried out using non-parametric tests (Wilcoxon test, Kruskal-Wallis test).

The GIN species were divided into 3 categories according to the species identified in RT-PCR: '*Haemonchus contortus*', '*Teladorsagia circumcincta*', '*Trichostrongylus colubriformis*'. The proportions of each species were compared at D0 and D21 by χ^2 test for each trial and for the global analysis, composed with the mean of proportion of each species. χ^2 tests were corrected when necessary with Yates correction (at least one theoretical number less than 5).

Results

The mean FEC (tannins and control groups pooled) at D0 and D21 for the global experiment or for each individual trial are presented in Fig. 1. Intensities of egg excretions at the beginning of the different trials were variable: in trial D, the mean FEC was relatively low (166 EPG), while in trial C, high excretions were noticed (2167 EPG). At D21, we noticed an increase of mean FEC in each trial.

The effect of supplementation was evaluated by comparing the FEC at D0 (beginning of the indoors period and the cure) and D21 (end of the cure) between the 2 groups (control vs tannin) of ewes (Fig. 2). At D0, there was no significant difference in the intensity of GIN egg excretions between the 2 groups of supplementation, whatever the trial and for the global analysis [$P = 0.55$, confidence interval (CI) (–600 to 302)]. Moreover, no significant difference between FEC of the 2 groups was reported at the end of the trial (all P values > 0.3), whatever the trial including the global analysis ($P = 0.85$, CI [–1151 to 955]).

The proportions of GIN species (Fig. 3) in each treatment group and trial were investigated. Helminth communities revealed the presence of the 3 GIN species in all trials but in different proportions. Grossly, *H. contortus* was the dominant species in summer in farms 1 and 2 (trial A, Fig. 3B and trial C, Fig. 3D), excepted in farm 3. By contrast, *T. colubriformis* was the main species in farm 2 during winter. The results obtained in farm 3 (summer time) are interesting because *T. colubriformis* is the only species found at D0 but the relative importance of *H. contortus* is increasing from D0 to D21 (Fig. 3E). χ^2 tests performed to compare relative abundances of GIN species showed variable results according to the trial. At D0, helminth communities were significantly different between the control and tannin groups on the trial A ($\chi^2 = 11.2$; 2 d.f.; $P = 0.03$) and trial B ($\chi^2 = 54.18$; 2 d.f.; $P = 1.7 \times 10^{-12}$). At D21, only trial B ($\chi^2 = 6.53$; 2 d.f.; $P = 0.038$) and trial D ($\chi^2 = 48.2$,

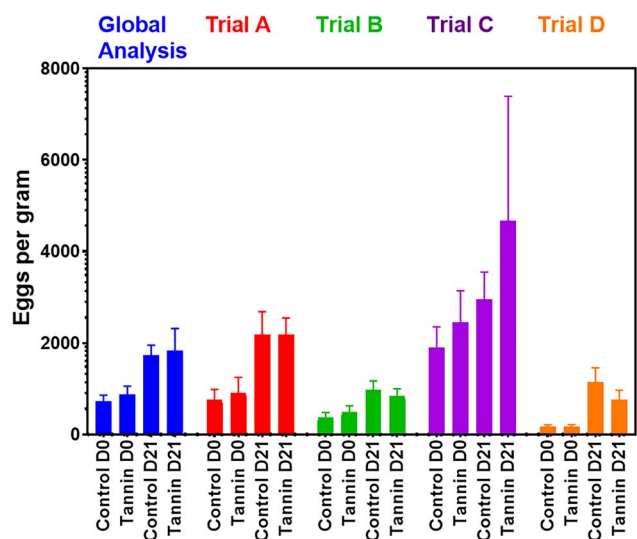


Figure 2. Intensities of egg excretions (Means and standard deviations) per group in the different trials and in pooled data.

2 df; $P = 3.4 \times 10^{-11}$) showed significant differences in helminth communities, characterized by an increased proportion of *T. colubriformis* and/or *T. circumcincta* in tannin group.

Discussion

This study evaluated the impact of a CTs-rich diet on FEC and as a possible alternative mode of control of GIN in commercial meat sheep farms. Commercially available pellets claiming anthelmintic activity were used at the recommended daily amount but for a double duration (21 days instead of 10 days). It is one of the few studies conducted on commercial flocks naturally infected with GIN and evaluating the effects of CTs supplementation on intensities of egg excretions and on the composition of helminth communities.

In this study, the use of pellets rich in condensed tannins was chosen over hay for several day-to-day management advantages. Pellets are easier to store and distribute to ewes, and their condensed tannin content is quality-assured by the manufacturer. In contrast, with healthy hay, this content can vary according to plant variety, number of harvest and drying (Kotze *et al.*, 2009; Azuhwi *et al.*, 2011, 2013; Malisch *et al.*, 2015), requiring farmers to measure the concentration of condensed tannins for each new harvest to adapt the distribution. In contrast, pellets are calibrated and dosed by the manufacturer, eliminating the need for recurrent analysis by the farmer.

The most striking result of this study was the absence of significant differences in FEC between ewes supplemented with tannins or lucerne in 4 trials. This result was obtained despite high intensities of GIN egg excretions (excepted for trial D at D0) and diversity in helminth communities. CTs have an effect on the installation and

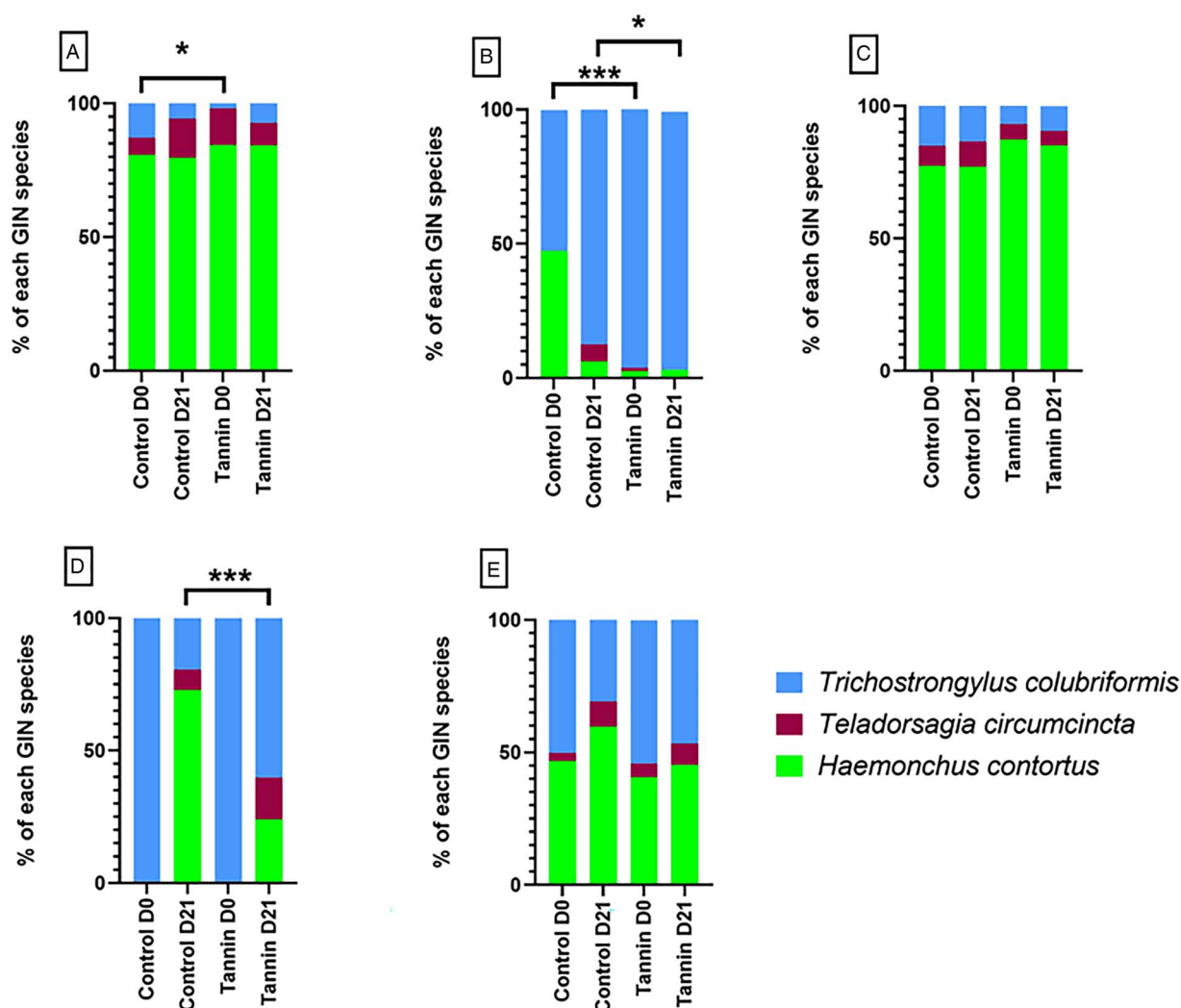


Figure 3. Relative abundances of the three gastrointestinal nematodes species (*Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*) determined by real-time PCR: (A) helminth community for trial A (summer), (B) helminth community for trial B (winter), (C) helminth community for trial C (summer), (D) helminth community for trial D (summer) and (E) global helminth community, with all trials are pooled. (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

fecundity of adult GIN in host (Paolini *et al.*, 2003b; Heckendorn *et al.*, 2006; Manolaraki *et al.*, 2010; Gujja *et al.*, 2013; Gaudin *et al.*, 2016). However, the direct evaluation of these parameters was not possible in our case, which led to the use of FEC as an indirect measure. Nonetheless, this measurement accurately represents the infection of animals by GIN (Cabaret *et al.*, 1998) and the pastures contamination. Recently, impact of CTs on FEC have been studied also in horses and rabbits with pellets formulations. In both cases, CT-rich diet with pellets didn't show any effect on FEC (Legendre *et al.*, 2017; Malsa *et al.*, 2022). Interestingly, in Malsa and colleagues' study, healthy-hay pellets were from the same manufacturer and the same variety than in our study.

One of the major concern raised by the use of pellets is the influence of the technological transformation process (dehydration) on condensed tannins and the conservation of their properties. The majority of *in vivo* studies have focused on the use of tannin-rich plants as hay or directly as polyphenolic extracts (Paolini *et al.*, 2004, 2005; Hoste *et al.*, 2006; Manolaraki *et al.*, 2010), particularly with healthy hay and/or quebracho, the component of the pellet tested in our study. Results of the activity of condensed tannins in the pellets seems linked with the temperature of the pelleting process: Legendre and colleagues (Legendre *et al.*, 2017), showed reduced protein precipitation activity of CTs *in vitro* with a temperature of pelleting process around 80°C, while Terrill and colleagues (Terrill *et al.*, 2007) do not exceed 70°C during the transformation process. In our case, the pellets tested came from the same manufacturer than in Legendre and colleagues study (Legendre *et al.*, 2017), so there may also be a problem of reduced activity of CTs in this formulation. Moreover, the effect of temperature during processing has only been tested on CTs from healthy hay, so the effects on CTs contained in quebracho pellets are not known.

In addition to studying the impact of CTs on FEC, we were also interested in their impact on GIN communities and, in particular, on the proportions of the 3 main species found in French farms. In the present study, the main species were *H. contortus* and *T. colubriformis* which is rather expected in the region: the humid and warm weather is favouring the *H. contortus* populations during spring and early summer, while the cold winters favour *T. colubriformis* populations. On *in vivo* experimentation, CTs have showed an impact on each of the 3 means species (Paolini *et al.*, 2003b; Heckendorn *et al.*, 2006), but the effect in co-infection were poorly investigated: in the horse assay of Malsa and colleagues (Malsa *et al.*, 2022), the nemabiome is not influenced by the CTs in the daily diet. In our case, helminth community were not systematically influence by CTs-rich diet, that suggest the effect is very weak.

The use of condensed tannins in the diet of small ruminants is of growing interest for the control of GIN, in a time of emergence of multiple anthelmintic resistance. While several studies have already showed a significant effect of condensed tannins on these parasites *in vitro* and in experimental infections, real-life trials with natural multi-species infections and carried out on commercial farms are much more unusual. In this study, we failed to demonstrate any effect of a pellet-based supplementation protocol for ewes on intensities of egg excretions nor in diversity of helminth communities. It is necessary to test new protocols on commercial farms in order to optimize the use of condensed tannins, particularly with regard to technical parameters such as cost, concentration of condensed tannins in diet, industrial process and the frequency and length of cure, before providing an effective and accessible control strategy to sheep breeders.

Data availability statement. Data available on request / reasonable request.

Acknowledgements. The authors would like to thank the breeders for their interest in the project, their feedback, their collaboration and the availability of their animals.

Author contributions. LB, GB, LS, CG, GGM and PJ developed the study design, selected volunteer farmers and set up the animal group. LB, CS, AC, SL, MG and LS distributed feed supplements, monitored animal health and took fecal samples. LB, CG and PJ performed coprological analyses, RT-PCR and statistical analyses

All authors participated in the writing and correction of this article and approve its content.

Financial support. The authors would like to thank the Nouvelle-Aquitaine region for funding this study, as part of the 'PARALUT' regional project, aimed at optimizing alternative control methods to anthelmintics in the management of GIN.

Competing interests. The authors declare no conflicts of interest.

Ethical standards. Stool collection are a part of routine veterinary procedures without any traumatic method. Such procedures are not qualified as animal experimentation involving vertebrates according to French laws, so no specific ethical clearance was required.

References

- Aguerre S, Jacquet P, Brodier H, Bournazel JP, Grisez C, Prévot F, Michot L, Fidelle F, Astruc JM and Moreno CR (2018) Resistance to gastrointestinal nematodes in dairy sheep: genetic variability and relevance of artificial infection of nucleus rams to select for resistant ewes on farms. *Veterinary Parasitology* **256**, 16–23.
- Ahuir-Baraja AE, Cibot F, Llobat L and Garijo MM (2021) Anthelmintic resistance: is a solution possible? *Experimental Parasitology* **230**, 1–7.
- Athanasiadou S, Kyriazakis I, Jackson F and Coop RL (2001) Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Veterinary Parasitology* **99**, 205–219.
- Azuñwi BN, Boller B, Martens M, Dohme-Meier F, Ampuero S, Günter S, Kreuzer M and Hess HD (2011) Morphology, tannin concentration and forage value of 15 Swiss accessions of sainfoin (*Onobrychis viciifolia* Scop.) as influenced by harvest time and cultivation site: morphology, tannin concentration and forage value of sainfoin. *Grass and Forage Science* **66**, 474–487.
- Azuñwi BN, Boller B, Dohme-Meier F, Hess HD, Kreuzer M, Stringano E and Mueller-Harvey I (2013) Exploring variation in proanthocyanidin composition and content of sainfoin (*Onobrychis viciifolia*): accession, harvest time and site effects on PA composition. *Journal of the Science of Food and Agriculture* **93**, 2102–2109.
- Bordes L, Dumont N, Lespine A, Souil E, Sutra J-F, Prévot F, Grisez C, Romanos L, Dailledouze A and Jacquet P (2020) First report of multiple resistance to eprinomectin and benzimidazole in *Haemonchus contortus* on a dairy goat farm in France. *Parasitology International* **76**, 1–7.
- Borgsteede FHM, Derksen DD and Huijbers R (2007) Doramectin and albendazole resistance in sheep in the Netherlands. *Veterinary Parasitology* **144**, 180–183.
- Brunet S, Aufrère J, El Babili F, Fouraste I and Hoste H (2007) The kinetics of exsheathment of infective nematode larvae is disturbed in the presence of a tannin-rich plant extract (sainfoin) both *in vitro* and *in vivo*. *Parasitology* **134**, 1253–1262.
- Brunet S, Fourquaux I and Hoste H (2011) Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (*Onobrychis viciifolia*) extract. *Parasitology International* **60**, 419–424.
- Cabaret J, Gasnier N and Jacquet P (1998) Faecal egg counts are representative of digestive-tract strongyle worm burdens in sheep and goats. *Parasite* **5**, 137–142.
- Cazajous T, Prevot F, Kerbiriou A, Milhes M, Grisez C, Tropee A, Godart C, Aragon A and Jacquet P (2018) Multiple-resistance to ivermectin and benzimidazole of a *Haemonchus contortus* population in a sheep flock from mainland France, first report. *Veterinary Parasitology: Regional Studies and Reports* **14**, 103–105.
- Chartier C, Pors I, Hubert J, Rocheteau D, Benoit C and Bernard N (1998) Prevalence of anthelmintic resistant nematodes in sheep and goats in Western France. *Small Ruminant Research* **29**, 33–41.
- Falzon LC, O'Neill TJ, Menzies PI, Peregrine AS, Jones-Bitton A, van Leeuwen J and Mederos A (2014) A systematic review and meta-analysis of factors associated with anthelmintic resistance in sheep. *Preventive Veterinary Medicine* **117**, 388–402.
- Gaudin E, Simon M, Quijada J, Schelcher F, Sutra J-F, Lespine A and Hoste H (2016) Efficacy of sainfoin (*Onobrychis viciifolia*) pellets against multi-resistant *Haemonchus contortus* and interaction with oral ivermectin: implications for on-farm control. *Veterinary Parasitology* **227**, 122–129.

- Geurden T, Hoste H, Jacquet P, Traversa D, Sotiraki S, Frangipane di Regalbano A, Tzanidakis N, Kostopoulou D, Gaillac C, Privat S, Giangaspero A, Zanardello C, Noé I, Vanimisetti B and Bartram D (2014) Anthelmintic resistance and multidrug resistance in sheep gastro-intestinal nematodes in France, Greece and Italy. *Veterinary Parasitology* **201**, 59–66.
- GraphPad Software (2023) *GraphPad Prism 8.0.1*. San Diego, California, USA: GraphPad Software.
- Gujja S, Terrill TH, Mosjidis JA, Miller JE, Mechineni A, Kommuru DS, Shaik SA, Lambert BD, Cherry NM and Burke JM (2013) Effect of supplemental sericea lespedeza leaf meal pellets on gastrointestinal nematode infection in grazing goats. *Veterinary Parasitology* **191**, 51–58.
- Heckendorn F, Häring DA, Maurer V, Zinsstag J, Langhans W and Hertzberg H (2006) Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* and *Cooperia curticiei* in lambs. *Veterinary Parasitology* **142**, 293–300.
- Heckendorn F, Häring DA, Maurer V, Senn M and Hertzberg H (2007) Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticiei*. *Veterinary Parasitology* **146**, 123–134.
- Hoste H, Jackson F, Athanasiadou S, Thamsborg SM and Hoskin SO (2006) The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends in Parasitology* **22**, 253–261.
- Jouffroy S, Bordes L, Grisez C, Sutra JE, Cazajous T, Lafon J, Dumont N, Chastel M, Vial-Novella C, Achard D, Karembe H, Devaux M, Abbadie M, Delmas C, Lespine A and Jacquet P (2023) First report of eprinomectin-resistant isolates of *Haemonchus contortus* in 5 dairy sheep farms from the Pyrénées Atlantiques département in France. *Parasitology* **150**, 365–373.
- Kommuru DS, Barker T, Desai S, Burke JM, Ramsay A, Mueller-Harvey I, Miller JE, Mosjidis JA, Kamisetti N and Terrill TH (2014) Use of pelleted sericea lespedeza (*Lepedeza cuneata*) for natural control of coccidia and gastro-intestinal nematodes in weaned goats. *Veterinary Parasitology* **204**, 191–198.
- Kommuru DS, Whitley NC, Miller JE, Mosjidis JA, Burke JM, Gujja S, Mechineni A and Terrill TH (2015) Effect of sericea lespedeza leaf meal pellets on adult female *Haemonchus contortus* in goats. *Veterinary Parasitology* **207**, 170–175.
- Kotze AC, O'Grady J, Emms J, Toovey AF, Hughes S, Jessop P, Bennell M, Vercoe PE and Revell DK (2009) Exploring the anthelmintic properties of Australian native shrubs with respect to their potential role in livestock grazing systems. *Parasitology* **136**, 1065–1080.
- Krücken J, Ehnert P, Fiedler S, Horn F, Helm CS, Ramünke S, Bartmann T, Kahl A, Neubert A, Weiher W, Daher R, Terhalle W, Klabunde-Negatsch A, Steuber S and von Samson-Himmelstjerna G (2024) Faecal egg count reduction tests and nemabiome analysis reveal high frequency of multi-resistant parasites on sheep farms in north-east Germany involving multiple strongyle parasite species. *International Journal for Parasitology* **25**, 1–14.
- Legendre H, Hoste H and Gidenne T (2017) Nutritive value and anthelmintic effect of sainfoin pellets fed to experimentally infected growing rabbits. *Animal: An International Journal of Animal Bioscience* **11**, 1464–1471.
- MAAF (1986) *Manuel of Veterinary Parasitological Laboratory Techniques*. Ministry of Agriculture, Fisheries and Food. London: HMSO.
- Malisch CS, Lüscher A, Baert N, Engström MT, Studer B, Frygasas C, Suter D, Mueller-Harvey I and Salminen J-P (2015) Large variability of proanthocyanidin content and composition in sainfoin (*Onobrychis viciifolia*). *Journal of Agricultural and Food Chemistry* **63**, 10234–10242.
- Malsa J, Courtot É, Boisseau M, Dumont B, Gombault P, Kuzmina TA, Basiaga M, Lluch J, Annonay G, Dhorne-Pollet S, Mach N, Sutra J-F, Wimel L, Dubois C, Guégnard F, Serreau D, Lespine A, Sallé G and Fleurance G (2022) Effect of sainfoin (*Onobrychis viciifolia*) on cyathostomine eggs excretion, larval development, larval community structure and efficacy of ivermectin treatment in horses. *Parasitology* **149**, 1439–1449.
- Manolaraki F, Sotiraki S, Stefanakis A, Skampardonis V, Volanis M and Hoste H (2010) Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. *Parasitology* **137**, 685–696.
- Mavrot F, Hertzberg H and Torgerson P (2015) Effect of gastro-intestinal nematode infection on sheep performance: a systematic review and meta-analysis. *Parasites & Vectors* **8**, 557–568.
- Milhes M, Guillermin M, Robin M, Eichstadt M, Roy C, Grisez C, Prévot F, Liénard E, Bouhsira E, Franc M and Jacquet P (2017) A real-time PCR approach to identify anthelmintic-resistant nematodes in sheep farms. *Parasitology Research* **116**, 909–920.
- Mueller-Harvey I, Bee G, Dohme-Meier F, Hoste H, Karonen M, Kölliker R, Lüscher A, Niderkorn V, Pellikaan WF, Salminen J-P, Skot L, Smith LMJ, Thamsborg SM, Totterdell P, Wilkinson I, Williams AR, Azuhni BN, Baert N, Brinkhaus AG, Copani G, Desrues O, Drake C, Engström M, Frygasas C, Girard M, Huyen NT, Kempf K, Malisch C, Mora-Ortiz M, Quijada J, Ramsay A, Ropiak NM and Waghorn GC (2019) Benefits of condensed tannins in forage legumes fed to ruminants: importance of structure, concentration, and diet composition. *Crop Science* **59**, 861–885.
- Paolini V, Bergeaud J, Grisez C, Prevot F, Dorchie P and Hoste H (2003a) Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Veterinary Parasitology* **113**, 253–261.
- Paolini V, Frayssines A, De La Farge F, Dorchie P and Hoste H (2003b) Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Veterinary Research* **34**, 331–339.
- Paolini V, Fouraste I and Hoste H (2004) *In vitro* effects of three woody plant and sainfoin extracts on 3rd-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology* **129**, 69–77.
- Paolini V, Prevot F, Dorchie P and Hoste H (2005) Lack of effects of quebracho and sainfoin hay on incoming third-stage larvae of *Haemonchus contortus* in goats. *The Veterinary Journal* **170**, 260–263.
- Papadopoulos E (2008) Anthelmintic resistance in sheep nematodes. *Small Ruminant Research* **76**, 99–103.
- Papadopoulos E, Gallidis E and Ptochos S (2012) Anthelmintic resistance in sheep in Europe: a selected review. *Veterinary Parasitology* **189**, 85–88.
- Paraud C, Marcotty T, Lespine A, Sutra JE, Pors I and Devos I (2016) Cross-resistance to moxidectin and ivermectin on a meat sheep farm in France. *Veterinary Parasitology* **226**, 88–92.
- Posit Team (2022) *RStudio: Integrated Development Environment for R*. Boston, MA: Posit Software.
- Raynaud J-P, William G and Brunault G (1970) Etude de l'efficacité d'une technique de coproscopie quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Annales de Parasitologie Humaine et Comparée* **45**, 321–342.
- R core Team (2023) *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.
- Ruiz-Huidobro C, Sagot L, Lugagne S, Huang Y, Milhes M, Bordes L, Prévot F, Grisez C, Gautier D, Valadier C, Sautier M and Jacquet P (2019) Cell grazing and *Haemonchus contortus* control in sheep: lessons from a two-year study in temperate Western Europe. *Scientific Reports* **9**, 12699.
- Sargison ND, Jackson F, Bartley DJ, Wilson DJ, Stenhouse LJ and Penny CD (2007) Observations on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. *Veterinary Parasitology* **145**, 65–76.
- Scheuerle MC, Mahling M and Pfister K (2009) Anthelmintic resistance of *Haemonchus contortus* in small ruminants in Switzerland and Southern Germany. *Wiener klinische Wochenschrift* **121**, 46.
- Stafford HA and Lester HH (1980) Procyanidins (Condensed Tannins) in green cell suspension cultures of Douglas fir compared with those in strawberry and avocado leaves by means of C₁₈-reversed-phase chromatography. *Plant Physiology* **66**, 1085–1090.
- Taylor MA, Learmount J, Lunn E, Morgan C and Craig BH (2009) Multiple resistance to anthelmintics in sheep nematodes and comparison of methods used for their detection. *Small Ruminant Research* **86**, 67–70.
- Terrill TH, Mosjidis JA, Moore DA, Shaik SA, Miller JE, Burke JM, Muir JP and Wolfe R (2007) Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. *Veterinary Parasitology* **146**, 117–122.
- Thorne JW, Redden R, Bowdridge SA, Becker GM, Khilji SF, Xie S, Bentley KL and Murdoch BM (2024) Reducing faecal egg count through selective breeding alters dorper lamb response to *Haemonchus contortus* in an artificial challenge trial. *Veterinary Parasitology* **308**, 1–13.
- Traversa D, Paoletti B, Otranto D and Miller J (2007) First report of multiple drug resistance in trichostrongyles affecting sheep under field conditions in Italy. *Parasitology Research* **101**, 1713–1716.
- Van den Brom R, Moll L, Borgsteede FHM, Van Doorn DCK, Lievaart-Peterson K, Dercksen DP and Vellema P (2013) Multiple anthelmintic resistance of *Haemonchus contortus*, including a case of moxidectin resistance, in a Dutch sheep flock. *Veterinary Record* **173**, 552–552.
- Vernerova E, Vondrova R, Kisova H, Svobodova V and Hera A (2009) Detection of benzimidazole resistance in gastrointestinal nematode parasites of sheep in the Czech Republic. *Veterinární Medicina* **54**, 467–472.
- Voigt K, Scheuerle M and Hamel D (2012) Triple anthelmintic resistance in *Trichostrongylus* spp. in a German sheep flock. *Small Ruminant Research* **106**, 30–32.