THÈSE

En vue de l'obtention du

DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :

Institut National Polytechnique de Toulouse (INP Toulouse)

Discipline ou spécialité :

Pathologie, Toxicologie, Génétique et Nutrition

Présentée et soutenue par :

Mme JESSICA QUIJADA PINANGO
le jeudi 17 décembre 2015

Titre :

RELATION STRUCTURE/ACTIVITE DE TANINS BIOACTIFS CONTRE LES NEMATODES GASTROINTESTINAUX (HAEMONCHUS CONTORTUS) PARASITES DES PETITS RUMINANTS

Ecole doctorale :
Sciences Ecologiques, Vétérinaires, Agronomiques et Bioingénieries (SEVAB)

Unité de recherche :
Interactions Hôtes - Agents Pathogènes (IHAP)

Directeur(s) de Thèse :
M. HERVÉ HOSTE

Rapporteurs :
M. ADIBE LUIZ ABDALLA, UNIVERSIDAD DE SAO PAULO
Mme HEIDI ENEMARK, NORWEGIAN VETERINARY INSTITUTE

Membre(s) du jury :
M. FRANÇOIS SCHELCHER, ECOLE NATIONALE VETERINAIRE DE TOULOUSE, Président
M. HERVÉ HOSTE, INRA TOULOUSE, Membre
Mme CARINE MARIE-MAGDELAINE, INRA PETIT BOURG, Membre
M. SMARO SOTIRAKI, HAO-DEMETER, Membre
M. VINCENT NIDERKORN, INRA CLERMONT FERRAND, Membre

Cette thèse est dédiée à mes parents, Teresa et Héctor,

À mon mari, Rafäel, pour son soutien inconditionnel, son amour illimité, sa patience, sa loyauté, son amitié et surtout sa confidence,

À ma grand-mère, Marcolina, car m'ait donné le plus grand et précieux cadeau en ma vie : ma foi en Dieu ma forteresse et mon espoir (Isaïas 41:13).

À mes adorés sœurs, belle- sœurs et frère : Yurlin, Indira, Iskay, Olga, Zoraida et Jesus. Merci pour l'amour infini que m'ont toujours été donné, celui qu'a été prolongé par l'amour de mes merveilleux neveux.

REMERCIEMENTS

Je remercie tout d'abord mon Dieu pour me donner le cadeau de la vie, et la forteresse pour vivre chaque jour.

Je tiens à remercier le Dr. Hervé Hoste de l'équipe « Interaction Tanins-Nématodes Digestifs » de l'UMR 1225 INRA/ENVT « Interactions Hôtes-Agents Pathogènes », pour avoir dirigé mes travaux de recherche. Je lui suis vraiment reconnaissante pour son aide, ses conseils et son soutien pendant toutes ces années. Je tiens à le remercier pour sa disponibilité, sa patience et gentillesse, ses conseils, bienveillance et la confiance qu'il m'a témoignée dès le premier jour. Je vous remercie de me donner l'opportunité de rejoindre l'équipe de recherche pendant ces trois années, elles representeront en temps précieux dans ma vie professionnelle mais aussi personnelle. Merci beaucoup, pour avoir été le meilleur directeur de thèse que je puisse avoir eu.

Je souhaite remercier le Pr. Adibe Luiz Abdalla de l' « Universidade de São Paulo » et le Dr. Heidi Enemark du « Norwegian Veterinary Institute » d'avoir accepté d'être rapporteurs de mon travail de thèse. Ainsi, je tiens à remercier le Dr. Carine Marie-Magdeleine de l'INRA Petit Bourg, le Dr. Vincent Niderkorn de l'INRA Clermont-Ferrand et le Dr. Smaragda Sotiraki de l'« HAO-DEMETER » d'avoir accepté d'être examinateurs de ma thèse.

J'adresse mes sincères remerciements au Pr. Nicolas Fabre de l'Université de Toulouse III Paul Sabatier et au Dr. Vincent Niderkorn de l'INRA Clermont-Ferrand, qui ont bien voulu faire partie de mon comité de thèse. Je les remercie pour leur intérêt pour mon travail, leurs conseils, disponibilité et leur appui permanent.

Cette étude a pu être réalisée grâce à l'appui et à la collaboration de nombreuses personnes, tant en France qu'à l'étranger. Je tiens donc également à remercier :

Le Pr. Juha-Pekka Salminen et la Pr. Maarit Karonen ensemble ainsi que leur équipe du travail pour m'avoir accueilli dans les laboratoires du « Natural Chemistry Research Group » de l'Université de Turku, Finlande. Merci pour m'avoir mené dans le fascinant monde des tanins. Je vous remercie tous pour votre aide, votre gentillesse, mais surtout l'inspiration et la motivation pendant mon stage dans cet extraordinaire pays.

La Pr. Irene Mueller-Harvey de la « School of Agriculture, Policy and Development» de l'« Université de Reading », pour son soutien constant et des précieux temps qu'elle m'a dédiée, ainsi que toute la sagesse qu'elle m'a donnée pendant mon stage dans son laboratoire. Merci à l'équipe constituée par : Christos Fryganas, Honorata Ropiak, Chaweewan Klongsiriwet, Parwin Majid (*RIP*), Mohammed Munis Dakheel, Christopher Drake et Prabhat Sakya pour toute l'aide, les conseils, et les bonheurs partagés pendant les deux mois où j'ai été avec eux.

Je voudrais aussi remercier tous mes collègues étudiants de thèse « ESR (Early Stage Researcher) » du projet « LegumePlus », pour toutes les discussions, nos voyages en commun au travers de cet important projet de recherche, pour les moments partagés au long des réunions du projet et les divers cours de formation faits ensemble.

Membres de l'équipe « Interaction Tanins-Nématodes Digestifs » présents au cours des trois dernières années, mes collègue et amis : Dr. Foteini Manolaraki, Dr. Blasius Azuhnwi, Dr. Aina Ramsay, Marine Rabeyrin, Audrey Torlotin, Lucie Legroux, Margaux Simon, Israel Chan-Pérez, Heloïse Legendre, Liza Bastide et M Eric Pardo. Très spécialement je voudrais remercier Elodie Gaudin, Ramzi El-Korso, Marica Engström et Liza Fonsou qui m'ont aide avec succès pour les études *in vivo* de cette thèse. Je tiens à vous remercier pour votre amitié et pour le temps passé ensemble à l'intérieur et à l'extérieur du laboratoire.

Le Pr. Michel Franc et le personnel du département de Parasitologie de l'Ecole Nationale Vétérinaire de Toulouse pour m'avoir accompagnée au quotidien pendant ces années. Merci pour la cordialité et la gentillesse de chaque jour.

Le Pr. François Schelcher et le personnel de l'UMR 1225 INRA/ENVT « Interactions Hôtes-Agents Pathogènes » pour leur sympathie et les services rendus et tout specialement d'avoir accepté d'être le président du jury de ma thèse.

Les secrétaires de l'UMR 1225 INRA/ENVT : Pascale Fauré, Marie Perrin, Corinne Fabreguettes et Laurence Lecomte pour leur amitié et leur aide au niveau administratif et la chaleureuse cordialité qu'elles m'ont toutes montrée dès le premier jour à l'INRA.

Les animaliers dont l'appui a été primordial pour la maintenance de nos animaux d'expérimentation, M Jean-Marc Delmas et toute l'équipe : Merci beaucoup!

Je remercie aussi tous mes amis et tous mes proches qui, de près ou de loin, m'ont soutenue durant ma vie mais aussi spécialement pendant ces années de thèse.

Enfin, notre travail de thèse a été réalisé grâce au soutien financier de : l'Union Européenne, grâce au projet Marie-Curie « LegumePlus » (Projet PITN-GA-2011-289377).

Je voudrais aussi remercier la France, ce pays qui m'a reçu à bras ouverts.

"A todos !Muchas gracias! estarán siempre en mi corazón".

(Merci beaucoup à tous, vous serez toujours dans mon cœur)

LISTE DES PUBLICATIONS

Les différents travaux de recherche menés au cours de cette thèse ont fait l'objet des publications suivantes :

Articles intégrés dans la thèse :

- Hoste, H., Torres-Acosta, J.F.J., **Quijada, J.**, Chan Pérez, I., Dakheel, M.M., Kommuru, D.S., Quijada, J., Mueller-Harvey, I., Terrill, T.H. Interactions between Nutrition and Infections with *H. contortus* (and related Gastrointestinal Nematodes) in Small Ruminants. *Adv. Parasit*. (accepté).

- **Quijada,** J., Fryganas, C., Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., Hoste, H. 2015. Anthelmintic activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from Small Ruminants are influenced by structural features of condensed tannins. *J. Agric. Food Chem.*, 63, 6346–6354.

- Klongsiriwet, C., **Quijada,** J., Williams, A.R., Mueller-Harvey, I., Williamson, E.M., Hoste, H. 2015. Synergistic inhibition of *Haemonchus contortus* exsheathment by flavonoid monomers and condensed tannins. *Int. J. Parasitol. Drugs Drug Resist.*, 5, 127–134.

LISTE DES ABREVIATIONS

ADD	Dérives d'amino-acetonitrile
ADN	Acide désoxyribonucléique
AH	Anthelminthique
BZs	Benzimidazoles
Da(s)	Daltons
C	Catéchol (catechin)
CE_{50}	Concentration efficace pour avoir le 50% d'effet
EC	Epicatéchol (epicatechin)
EGC	Epigallocatéchol (epigallocatechin)
EGCG	Epigallocatéchol gallaic (epigallocathechin gallate)
g	Gramme
GABA	Acide gamma aminobutyrique
GC	Gallocatéchol (gallocatechin)
GCG	Gallocatéchol gallaic (gallocathechin gallate)
GI(s)	Gastro-intestinal(aux)
GIN(s)	Nématodes gastro-intestinaux
HCl	Acide hydrochlorhydrique
HPLC	Chromatographie en phase liquide à haute performance
Kg	Kilogramme
L1s	Larves de stade 1
L2s	Larves de stade 2
L3s	Larves de stade 3
L4s	Larves de stade 4
LEIA	Méthode d'inhibition du dégainement larvaire
LMIA	Méthode d'inhibition de la migration larvaire
mDP	Degré moyen de polymérisation
MS	Matière sèche
OH	Hydroxyle
OPG	Œufs par gramme de fèces
PC	Procyanidol (procyanidin)
PD	Prodelphinidol (prodelphinidin)
PEG	Polyéthylène glycol
PM	Poids moléculaire
PS	Poids sec
PSMs	Métabolites secondaires des plantes
PRPs	Protéines riches en proline
PVPP	Polyvinyl polypyrrolidone
SGIs	Strongles gastro-intestinalaux
TCs	Tannins condensés
THs	Tannins hydrolysables
TR	Riche en tanin
TTs	Tannins totaux

TABLE DES MATIERES

RESUME

Les nématodes gastro-intestinaux (NGIs) représentent une contrainte majeure en élevage des ruminants. Jusqu'à présent, la maîtrise de ces parasitoses a reposé essentiellement sur l'emploi répété de traitements anthelminthiques (AH) de synthèse. Cependant, le développement constant de résistances aux AH dans les populations de nématodes chez les petits ruminants conduit à rechercher des méthodes alternatives ou complémentaires de lutte contre ces parasitoses. Au cours des deux dernières décennies, les données se sont accumulées montrant que certaines plantes étaient dotées de propriétés anthelminthiques, ce qui a été associé à la présence de tannins condensés (TCs) et de flavonoïdes. Ces ressources contenant des TCs, exploitées comme nutricaments, représentent donc une alternative attractive à la chimiothérapie, en combinant des effets bénéfiques sur la santé et la nutrition des ruminants. Toutefois, une des difficultés à résoudre, pour un usage pertinent de ces nutricaments, est de comprendre l'origine de la variabilité des résultats observés dans l'activité AH. A côté de facteurs quantitatifs, la qualité (la structure) des TCs semble également jouer un rôle dans les propriétés antiparasitaires mais ce facteur demeure peu exploré. En utilisant *Haemonchus contortus* comme modèle expérimental de nématode et le sainfoin (*Onobrychis viciifolia)* comme modèle de plantes contenant des tannins, l'objectif général de cette thèse a été de mieux comprendre les relations structure/activité des TCs. Les objectifs spécifiques des diverses études menées ont été **1)** d' examiner *in vitro* (par la méthode LEIA) l'activité AH de 36 fractions purifiées de TCs présentant une large diversité de caractéristiques structurelles [*ex:* la taille (mDP), les proportions prodelphinidols /procyanidols (PD/PC) et *trans/cis*], et **2)** le rôle possible d'interactions entre tannins et flavonoïdes, **3)** d' évaluer et comparer *in vivo* chez des agneaux, les conséquences sur les populations de vers et sur la résilience de l'hôte de la distribution de 2 ressources contenant des TCs de qualité contrastée pour les valeurs PD/PC [fort PD/PC, sainfoin (*O. viciifolia)*; bas PD/PC, pellicules de noisettes (*Corylus avellana*)], **4)** d' examiner si les résultats varient en fonction de l'espèce et de la localisation des parasites [abomasum (*H. contortus)* vs intestin grêle (*Trichostrongylus colubriformis*)], **5)** d'évaluer les concentrations, la qualité et la bio-disponibilité des TCs le long du tube digestif chez les ovins. Les résultats acquis ont montré: **1)** des relations *in vitro* entre certaines caractéristiques des TCs et l'activité AH : un rapport élevé PD/PC pour les 2 espèces de nématodes auquel s'ajoute un rôle de la taille des tanins (mDP) pour *H. contortus* sont les facteurs identifiés. De manière générale, *H. contortus* s'est avéré plus sensible (valeurs EC50 plus faibles) aux effets des diverses ressources que *T. colubriformis*. Des synergies d'effets AH ont été observées entre tannins et flavonoïdes (quercétine et

lutéoline), tout particulièrement dans le cas des TCs avec un faible rapport PD/PC. Dans l'étude *in vivo*, les agneaux consommant du sainfoin ont montré des effets AHs et une amélioration de la résilience de l'hôte alors que les effets de la noisette ont été beaucoup plus discrets. Les analyses menées sur les contenus des divers organes digestifs et les fèces, pour les deux types de ressources ont montré de fortes réductions de TCs détectés par rapport aux teneurs initiales dans la ration alors que les caractéristiques de structure étaient globalement préservées au long du tube digestif. En conclusion, le rôle de la qualité (structure) des TCs dans l'activité AH a été confirmé à la fois par les études *in vitro* et *in vivo.* Ces résultats suggèrent l'importance de prendre en compte ces facteurs dans la recherche de ressources pouvant être exploitées comme futurs nutricaments chez les ruminants.

ABSTRACT

Gastrointestinal nematodes (GINs) represent a major threat for the breeding and production of grazing ruminants. So far, their control has been based mainly on the repeated use of synthetic AH drugs. However, worm populations in small ruminants have consistently developed resistance against all AH drugs. In the search for alternative solutions to such drug treatments, the last two decades have provided evidence that some plants possess natural AH bioactivity. This has been related to the presence of condensed tannins (CTs) and flavonoids. Therefore, CT-containing resources represent a promising alternative to chemotherapy especially when used as nutraceuticals that combine beneficial effects on health and nutrition in small and large ruminants. However, one of the main constraint to use these nutraceuticals relates to the variations in AH results. The structural features (quality) that contribute to the AH activities of CTs remain elusive. By using the GIN *Haemonchus contortus* as an experimental model of nematode and *Onobrychis viciifolia* as a model of CT-containing plant, the general aim of this thesis was to better understand the structure/activity relationship of CT. The specific objectives were **1)** to examine *in vitro* (using LEIA) the AH activity of 36 purified CT fractions with a wide range of structural features [*i.e.* size (mDP), prodelphinidin/procyanidin (PD/PC) and *trans/cis* ratios], and **2)** the possible role of interactions between tannins and flavonoids, **3)** to evaluate *in vivo* the AH activity and host resilience of two CT-resources with contrasting PD/PC ratios (i.e. high PD/PC, *O. viciifolia*; low PD/PC, *Corylus avellana*), **4)** to examine whether these results vary depending on the parasites species (abomasal, *H. contortus* vs intestinal, *Trichostrongylus colubriformis*), **5)** to examine the CT availability (quantity and quality) along the digestive tract in sheep. In regard to CT features and AH activity, *in vitro* correlations were observed between AH activity and high PD/PC for both species. The mDP was an additional criteria for *H. contortus*. Lower EC50 values were assessed for *H. contortus*. Synergistic AH effects were observed between CT and flavonoids (quercetin and luteolin), and they were higher with fractions of low PD/PC ratios. *In vivo*, the lambs consuming sainfoin pellets showed consistently higher AH effects and improvement in the host's resilience. Analyses of digesta samples and faeces, for both diets showed large reduction of detected CT related to the feed, whereas the CT structures seemed preserved along the gut. In our studies, the role of CT structural features in the AH activity was observed both *in vitro* and *in vivo.* These results confirmed the influence of CT quality in the AH effects, and underline the relevance of taking these factors into account when screening CT resources as potential nutraceuticals in small ruminants.

RESUMEN

Los nematodes gastrointestinales (NGIs) son una importante limitante en la producción de rumiantes a pastoreo. Hasta ahora, su control ha estado basado principalmente en la administración repetida de fármacos antihelmínticos (AH) de síntesis. Sin embargo, las poblaciones de vermes en pequeños rumiantes han desarrollado consistentemente resistencia contra todas las drogas AH. En la búsqueda por soluciones alternativas a los tratamientos con tales drogas, en las últimas dos décadas se ha acumulado evidencia de que ciertas plantas poseen bioactividad AH natural. Esta actividad, ha sido relacionada con la presencia de taninos condensados (TC) y flavonoides. Por lo tanto, los recursos forrajeros que contienen TC representan una prometedora alternativa a la quimioterapia AH, especialmente cuando son usados como nutracéuticos, que combinan efectos beneficiosos sobre la salud y la nutrición en pequeños y grandes rumiantes. No obstante, una de las principales limitaciones en el uso de tales nutracéuticos se refiere a las variaciones en los resultados AH. Las características estructurales (calidad) de los TCs que contribuyen a las actividades AH están poco definidas. Usando el NGI *Haemonchus contortus* como modelo experimental de nematode y a *Onobrychis viciifolia* como modelo de planta que contiene TC, el objetivo general de esta tesis fue la mejor comprensión de la relación estructura/actividad de los TCs. Los objetivos específicos fueron: **1)** examinar *in vitro* (LEIA) la actividad AH de 36 fracciones purificadas de TCs con amplio rango de valores de características estructurales [*i.e.* tamaño (mDP); proporción de prodelfinidina/procianidina (PD/PC) y *trans/cis*], y **2)** el posible papel de las interacciones entre taninos y flavonoides, **3)** evaluar *in vivo* la actividad AH y sobre la resiliencia del hospedador de dos recursos que contienen TCs con diferente calidad [proporción de PD/PC (*i.e. O. viciifolia*, con alto contenido de PD; *Corylus avellana* con bajo contenido de PD)], **4)** examinar si tales resultados varían dependiendo de la especie de parásitos (el abomasal, *H. contortus* vs. el intestinal *Trichostrogylus colubriformis*), **5)** examinar la disponibilidad de TC (en cuanto a cantidad y calidad) a lo largo del tracto digestivo de ovinos. En relación con las características estructurales de los TCs y su actividad AH, se observaron correlaciones entre la actividad AH y una alta proporción de PD para ambas especies de parásito. El mDP fue un criterio adicional para *H. contortus*. Menores valores de EC50 fueron calculados para *H. contortus*. Efectos sinérgicos fueron observados entre TC y flavonoides (quercetina y luteolina), y fueron más fuertes con fracciones con baja proporción de PD/PC. *In vivo*, los corderos que consumieron granulado de sainfoin, mostraron consistentemente mayores efectos AH y mejora en la resiliencia del hospedador. Los análisis de muestras de contenido digestivo y heces, para ambas dietas, mostraron

una gran reducción en la cantidad de TC en relación con la del alimento, mientras que la estructura (calidad) de los TCs parece conservada a lo largo del tubo digestivo. En nuestros estudios, el rol de las características estructurales de los TCs en la actividad AH fue observado tanto *in vitro* como *in vivo*. Estos resultados confirman la influencia de la calidad de los TCs y subraya la importancia de considerar estos factores en la selección de recursos que contienen TC como potenciales nutracéuticos en pequeños rumiantes.

INTRODUCTION

1. Les nématodes gastro-intestinaux des ruminants

1.1. Généralités sur les nématodes gastro-intestinaux

Chez les ruminants, les nématodes parasites du tractus digestif les plus prévalents, communément appelés strongles gastro-intestinaux (GIs) ou strongles digestifs en médecine vétérinaire, appartiennent à l'ordre des Strongylidea et à deux familles distinctes: les Trichostrongyloidea (principaux genres : *Teladorsagia*, *Cooperia*, *Trichostrongylus* et *Haemonchus*) et les Strongyloidea (genre: *Oesophagostomum*) (Urquhart et al., 1996) (Tableau 1).

Tableau 1. Principales espèces de trichostrongyles des ruminants et leur localisation dans le tractus digestif

Sous-familles	Espèces	Localisation chez l'hôte	hôtes
Haemonchinae	*Haemonchus contortus*	Abomasum	Ovins, Caprins
	Haemonchus placei		Bovins
	Haemonchus longistipes		Dromadaires
Trichostrongylinae	*Trichostrongylus colubriformis*	Intestin Grêle	Ovins, Caprins, Bovines
	Trichostrongylus axei	Abomasum	Ovins, Caprins, Bovines
	Trichostrongylus vitrinus	Intestin Grêle	Ovins, Caprins
	Trichostrongylus capricola	Intestin Grêle	Ovins, Caprins
Ostertagiinae	*Teladorsagia circumcincta*	Abomasum	Ovins, Caprins
	Ostertagia ostertagi	Abomasum	Bovins
	Ostertagia occidentalis	Abomasum	Ovins
	Ostertagia trifurcata	Abomasum	Ovins, Caprins
Cooperiinae	*Cooperia curticei*	Intestin Grêle	Ovins, Caprins
	Cooperia oncophora	Intestin Grêle	Bovins
	Cooperia punctata	Intestin Grêle	Bovins
Nematodirinae	*Nematodirus abnormalis*	Intestin Grêle	Ovins, Caprins, Dromadaires
	Nematodirus battus		Ovins, Caprins
	Nematodirus helvetianus		Bovins
	Nematodirus spathiger		Ovins, Caprins, Bovins
	Nematodirus filicollis		Ovins, Caprins
Oesophagostominae	*Oesophagostomum columbianum*	Caecum et Colon	Ovins, Caprins
	Oesophagostomum radiatum		Bovins
	Oesophagostomum venulosum		Ovins, Caprins
Chabertiinae	*Chabertia ovina*	Colon	Ovins, Caprins, Bovins

Les principales caractéristiques morphologiques de ces nématodes sont d'avoir une petit taille (4 – 35 mm), l'absence de capsule buccale (ou très rudimentaire si présente), et pour les mâles la présence d'un genitalia (d'une bourse caudale) bien développé à l'extrémité postérieure (Urquhart et al., 1996).

Les strongyloses GIs sont souvent dues à plusieurs espèces parasites (c'est-à-dire des infestations mixtes) dans les divers organes du tube digestif.

Les nématodes GIs ont une distribution mondiale avec des prédominances variables d'espèce selon les grandes zones climatiques. Ainsi, en zones tempérées, les trichostrongles les plus fréquemment retrouvés dans les élevages de petits ruminants au pâturage sont *Telardorsagia circumcincta* (espèce abomasale) et *Trichostrongylus colubriformis* (espèce intestinale) (Etter et al., 2000 ; O'connor et al., 2006). *Haemonchus contortus*, une autre espèce de l'abomasum, est moins fréquente dans les élevages des zones tempérées.

Par ailleurs, en zones tropicales et subtropicales, *H. contortus* est très largement réparti et très pathogène (O'connor et al., 2006 ; Urquhart et al., 1996). *T. colubriformis* est également retrouvé dans les élevages des régions tropicaux (Chartier et al., 2000) ainsi qu' *Oesophagostomum columbianum*.

1.2. Cycle biologique

Le cycle biologique des nématodes GIs des ruminants est monoxène (un seul hôte, l'hôte définitif). Il comprend deux phases : une phase libre dans le milieu extérieur (ou phase exogène) et une phase parasitaire chez l'hôte (ou phase endogène) (Urquhart et al., 1996) (Figure 1).

- ✓ La phase libre

La phase exogène du cycle des nématodes GIs débute avec l'élimination des œufs pondus par les vers femelles dans les fèces de l'hôte. Les œufs sont ainsi répandus sur les prairies. Lorsque les conditions environnementales sont favorables (température minimale de 10°C et taux d'humidité de 60%), les œufs s'embryonnent et éclosent, libérant des larves de stade 1 (L1s) (Urquhart et al., 1996). Après l'éclosion, les L1s évoluent au travers de deux mues pour devenir des larves infestantes (L3s). Les stades intermédiaires L1 et L2 sont peu résistants dans le milieu extérieur contrairement aux œufs et aux L3s. En fonction des conditions environnementales, les L3s peuvent survivre sur un pâturage plusieurs mois en zones tempérées grâce à leurs réserves lipidiques, alors qu'en zones tropicales ou subtropicales, la survie des L3s est de quelques semaines seulement (O'connor et al., 2006 ; Urquhart et al., 1996 ; Wang et al., 2014).

- ✓ La phase parasitaire

La phase parasitaire commence par l'ingestion des L3s par l'hôte lors du pâturage. Dans le tube digestif, les L3s se libèrent d'abord de leur gaine (dégainement), ce qui marque la transition entre vie libre et vie parasitaire (De Rosa et al., 2005 ; Hertzberg et al., 2002).

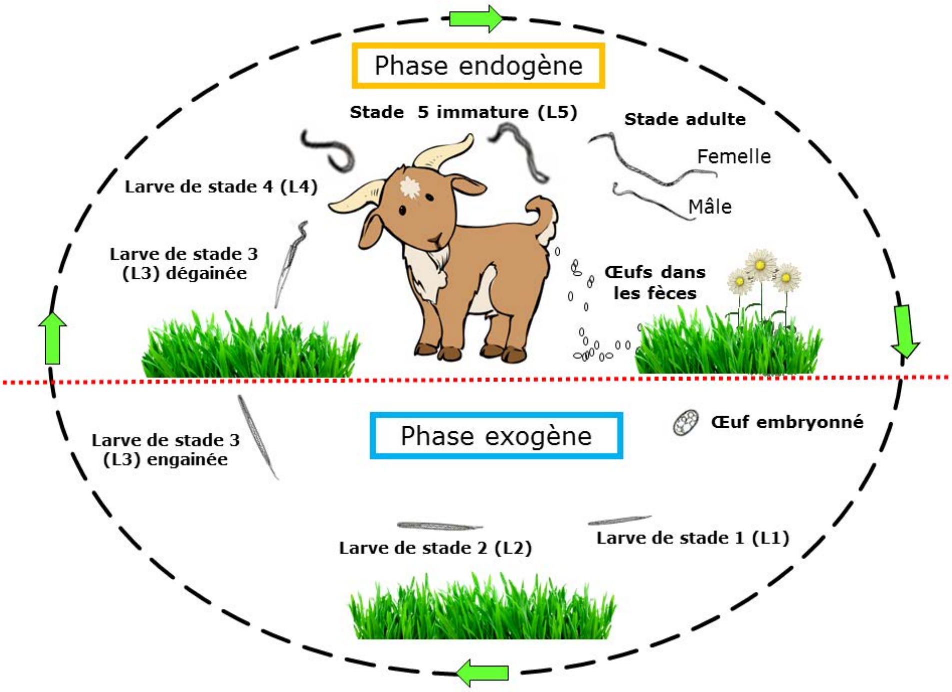


Figure 1. Cycle biologique des nématodes (Nematoda : Strongylida) gastro-intestinaux des ruminants.

Les L3s dégainées pénètrent ensuite dans la muqueuse digestive où elles muent en larves 4 (L4s). Les L4s muent une dernière fois pour donner le stade 5, également appelé stade préadulte ou juvénile. Le passage au stade adulte correspond à l'acquisition de la maturité sexuelle. Après fécondation, les femelles pondent des œufs excrétés dans les matières fécales de l'hôte (Urquhart et al., 1996). En hiver ou durant une longue période sèche, il est fréquent que les L4s s'enkystent dans la muqueuse digestive retardant ainsi leur développement (phénomène d'hypobiose larvaire) et reprennent leur évolution au printemps ou à la saison des pluies suivante (Chartier et al., 2000).

La chronologie du développement des stades parasites des nématodes gastro-intestinaux (GIs) diffère entre les espèces, de l'importance de l'infestation et de l'hôte (résistance). Le temps écoulé entre l'ingestion des L3s par l'hôte et la première ponte d'œuf par les vers est appelée la période prépatente. En l'absence d'hypobiose larvaire, cette période dure généralement de 2 à 3 semaines pour la plupart des espèces parasites chez les ovins et les caprins. Exceptionnellement, elle dure jusqu'à 5 semaines pour certaines espèces Strongyloidea chez les bovins, telles que *Oesophagostomum radiatum* (Urquhart et al., 1996).

1.2.1. Biologie des larves infestantes

Au cours des phases exogène puis endogène du cycle biologique des nématodes, les larves du troisième stade (L3) sont soumises à diverses conditions environnementales. Ces larves infestantes ont développé des adaptations physiologiques pour ces deux situations. Ainsi, sur le pâturage, les L3s des nématodes GIs possèdent une gaine (ou exuvie), vestige de la cuticule de la L2. En raison de cette gaine, les L3s sont très résistantes aux conditions extérieures (O'connor et al., 2006). La durée de la survie des L3s dépend de l'espèce, des conditions climatiques et de leur localisation dans le milieu. Pour *H. contortus*, les L3s subsistent de 10 à 15 semaines au printemps et seulement 3 à 4 semaines pendant un été chaud et sec. Les mousses et les matières fécales offrent des conditions optimales de survie pour les L3s en créant des microclimats favorables (O'connor et al., 2006 ; Wang et al., 2014).

Les L3s sont également très résistantes aux agents chimiques et aux agents biologiques. Néanmoins, elles sont naturellement en concurrence vitale avec d'autres organismes, tels certaines bactéries ou certains champignons microscopiques (Paraud, 2006). Les L3s sont très mobiles. Elles se déplacent horizontalement et verticalement sur l'herbe suivant un hygrotropisme positif (recherche d'humidité), un phototropisme négatif (fuite d'une trop forte lumière) et selon un géotropisme négatif (élévation au-dessus du sol) (Oliveira et al., 2009 ; Wang et al., 2014). Cette mobilité les rend plus accessibles à l'ingestion par les hôtes et augmente leur chance de les parasiter. Néanmoins, ces mouvements peuvent être préjudiciables à leur survie car les L3s sont incapables de se nourrir et de renouveler leurs réserves (O'connor et al., 2006).

L'infestation de l'hôte par les L3s débute par le dégainement, c'est-à-dire la perte active de la gaine. Ce phénomène s'opère dans la portion du tube digestif qui précède l'organe où s'établissent les vers adultes (Hertzberg et al., 2002). Donc, les L3s des espèces abomasales se dégainent dans le rumen alors que les espèces intestinales se dégainent dans l'abomasum (Rahman et Collins, 1990 ; Rogers et Sommerville, 1963).

Le dégainement est un processus restreint dans le temps (Hertzberg et al., 2002 ; De Rosa et al., 2005). La majorité des larves d'*H.contortus* sont dégainées 60 à 80 minutes après leur ingestion par l'hôte (Hertzberg et al., 2002). Le dégainement est un phénomène actif en réponse à plusieurs stimuli tels l'exposition à un environnement riche en dioxyde de carbone, la valeur de pH (neutre pour *H.contortus* et acide pour *T. colubriformis*) ou la variation de température après ingestion (Rogers et Sommerville, 1968). Ces stimuli induisent la sécrétion par des cellules glandulaires de la L3 et l'excrétion par des pores excréteurs d'un fluide riche en protéases et acétylcholinestérases (Mallet et Lesage, 1987). Le dégainement s'opère ensuite en 3 étapes successives : 1) la formation d'un anneau indenté dans la partie antérieure de la gaine ; 2) la digestion et la séparation de la partie proximale du reste de la gaine; 3) la sortie de la L3 (Gamble et al., 1989 ; Rogers et Sommerville, 1963).

1.3. Epidémiologie des strongyloses gastro-intestinales

De manière globale, les infestations des ruminants par les nématodes GIs dépendent de la conduite des animaux au pâturage puisque la phase de vie libre de ces strongles ne peut se dérouler que sur la prairie et que les ruminants s'infestent par les L3s présentes sur l'herbe (Chartier et al., 1992). Les animaux élevés à l'intérieur, sans contact avec des animaux ayant pâturé, et recevant du foin ou de l'ensilage, ne sont normalement pas infestés par des L3s (Chartier et al., 1992 ; Torres-Acosta et Hoste, 2008). Néanmoins, la distribution de fourrage frais contaminé peut être source potentielle d'infestation. La notion d'infestivité correspond à la quantité de L3s présentes sur une parcelle. L'infestivité dépend du nombre d'œufs déposés, de leur vitesse de développement et de la survie des L3s. L'intensité de l'infestation de l'hôte dépend du nombre de L3s ingérées mais aussi des caractéristiques de l'hôte (réceptivité).

1.3.1. Facteurs liés aux strongles

Les contacts entre l'hôte et les L3s sont à l'origine de l'infestation des animaux. Ils dépendent de la biologie des vers, du comportement des L3s et de celui des hôtes.

- ✓ Niveau d'excrétion d'œufs (prolificité parasitaire) :

La contamination des prairies est d'autant plus importante que l'excrétion des œufs de nématode par l'hôte l'est. Or, les espèces de trichostrongles digestifs n'ont pas la même prolificité (Chartier et al., 2000). Par comparaison aux femelles de *T. colubriformis* ou de *T. circumcincta,* qui pondent quelques centaines d'œufs par femelles et par jour, Les femelles d'*H.contortus* sont très prolifiques (5 000 à 10 000 œufs par femelle et par jour).

- ✓ Mobilité des L3s :

Les L3s des nématodes GIs sont très mobiles en phase liquide. Elles se déplacent sur l'herbe suivant un phototropisme négatif et un hygrotropisme positif (Rogers et Sommerville, 1963 ; Wang et al., 2014). Les deux moments qui favorisent le plus les infestations des hôtes sont tôt le matin et au crépuscule, lorsque l'ensoleillement est réduit et quand la rosée recouvre l'herbe (Smith et Sherman, 1994).

1.3.2. Facteurs liés à l'hôte

1.3.2.1. Comportement alimentaire de l'hôte

Deux principaux modes de comportements ont été décrits chez les 2 espèces de petits ruminants qui illustrent l'importance du comportement alimentaire pour expliquer les différences d'ingestion de L3s de nématodes Gis et donc de niveau d'infestation.

Pour des raisons liées au comportement des L3s, les risques d'infestation sont réduits lors de l'exploitation d'arbustes ou de buissons. A l'inverse, la consommation d'herbe est propice au contact avec les L3s. Ce constat expliquerait en partie que, sur parcours ou environnement pastoral, les chèvres, au comportement de cueilleur, sont généralement moins infestées que les moutons (comportement de brouteur) (Hoste et al., 2001 ; Hoste et al., 2010 ; Hoste et Chartier, 1998 ; Vercruysse, 1983). Par ailleurs, même chez les ruminants décrits comme des brouteurs, comme les ovins, des comportements acquis existent. L'évitement des zones riches en matières fécales semble un comportement très marqué chez les animaux parasités, qui conduit à réduire les infestations (Hutchings et al., 2003).

1.3.2.2. Réponses de l'hôte: résistance et résilience

La résistance est définie comme l'aptitude de l'hôte à mettre en place et maintenir des réponses qui modifient la biologie des vers en limitant leur installation, développement ou reproduction, ou en provoquant l'élimination de populations établies (Douch et al., 1996; Hoste et al., 2006). Le concept a été associé à l'existence et à la mise en place d'une réponse immunitaire.

La résilience est l'aptitude de l'hôte à supporter les effets pathologiques des infestations par les NGI et à maintenir la santé des animaux et la production zootechnique en dépit de ce parasitisme (Baker et al., 1998 ; Hoste et al., 2006 ; Van Houtert and Sykes, 1996). A l'inverse du phénomène de résistance, les mécanismes sous-jacents expliquant la résilience reste très peu explorés.

a) Mécanismes de l'immunité face aux NGIs

Deux types de réponses immunitaires (innée ou acquise) ont été évoqués pour expliquer la résistance de l'hôte aux infestations par les NGIs (Andronicos et al., 2010). La réponse innée correspond à l'aptitude de l'hôte à réguler les populations de vers par des mécanismes non-spécifiques tels que des particularités physiologiques (mouvements péristaltiques, pH gastrique, sécrétion de mucus) et une grande réactivité inflammatoire (phagocytose, système du complément, sécrétion de cytokines) (Guedes et al., 2010 ; Lacroux, 2006).

La réponse acquise fait suite à des infestations préalables. C'est une réponse plus tardive mais plus spécifique du nématode GI concerné. Chez les ovins et les caprins, cette réponse immunitaire serait associée au rôle des lymphocytes T de type TH2– CD4+ qui produisent des interleukines de type IL-4, IL-13, IL-5 et IL-9 (Karanu et al., 1997 ; Robinson et al., 2011) et des lymphocytes B (Pérez et al., 2001, 2008). Une mastocytose tissulaire, des taux élevés de globules leucocytes, une éosinophilie sanguine et tissulaire et une production accrue d'anticorps (majoritairement de type Ig1, IgA et IgE) ont également été décrits lors d'infestations par les trichostrongles GIs (Balic et al., 2000 ; Balic et al., 2002 ; Bambou et al., 2008 ; Lacroux, 2006 ; Pérez et al., 2003).

b) Facteurs de variation de la résistance de l'hôte :

✓ Age:

De manière générale, les jeunes animaux sont les plus sensibles et réceptifs aux infestations. Néanmoins, le facteur 'Age' serait plus discriminant chez les ovins et les bovins que chez les caprins (Urquhart et al., 1996). Ces différences de réceptivité selon l'âge seraient surtout liées à l'acquisition d'une immunité à la suite des infestations répétées (Chartier et al., 2000 ; Hoste et al., 1999; Tariq et al., 2008 ; Urquhart et al., 1996).

✓ Espèce animale :

L'espèce de l'hôte module la réceptivité aux nématodes GIs. Ainsi, à la suite d'infestations répétées par les trichostrongles GIs, la réponse immunitaire observée est moins efficace chez les caprins que chez les ovins et les bovins (Hoste et al., 1999 ; Smith et Sherman, 2009).

✓ Facteurs génétiques/ race et lignée :

Des différences inter-races et inter-lignées (intra-races) de résistance aux nématodes GIs ont été largement décrites chez les ovins (Bishop et Morris, 2007 ; Bricarello et al., 2005 ; Gauly et Erhardt, 2001 ; Shakya et al., 2011). Des différences de niveaux d'infestation entre races et lignées de chèvres ont également été mises en évidence (Bishop et Morris, 2007 ; Bricarello et al., 2005 ; Hoste et al., 2001; Vagenas et al., 2002). Cependant, les données chez les caprins sont contradictoires et l'origine des différences reste mal définie. Hoste et al. (2001) ont suggéré qu'à côté de la réponse immunitaire, le comportement alimentaire serait une composante importante à prendre en considération chez les caprins.

✓ Statut nutritionnel de l'hôte:

En raison des perturbations pathophysiologiques induites (baisses d'ingestion, maldigestion/ malabsorption dans le tube digestif, réorientations de métabolisme de l'hôte) (Hoste et al., 2005b ; Urquhart et al., 1996), les infestations par les NGIs peuvent être assimilées à une pathologie conduisant à une malnutrition chronique (Cf. Chapitre 1.4). En raison de l'importance des conséquences nutritionnelles des infestations, il est logique que la qualité ou la quantité de la ration offerte pour couvrir les besoins des ovins ou caprins sont considérées comme des facteurs majeurs influant sur les capacités de réponse aux infestations.

Ces deux volets des interactions entre nutrition de l'hôte et infestations pas les NGIs (en se focalisant sur le modèle *Haemonchus contortus*) ont fait l'objet d'une synthèse bibliographique qui est partie intégrante des travaux de cette thèse (Cf. Etude bibliographique).

✓ Statut physiologique :

Chez les brebis ou les chèvres en gestation, des augmentations d'excrétion fécale d'œufs de nématodes ont été couramment observées autour de la mise bas, le phénomène étant décrit comme le 'periparturient rise' où des variations endocrines en augmentent la susceptibilité aux strongles (Hoste et al., 1999; Kidane et al., 2010 ; Rahman and Collins, 1992). Ces pics d'excrétion seraient expliqués par à un « relâchement » du système immunitaire des chèvres ou brebis en fin de gestation et en début de lactation, lié à des déficits nutritionnels (surtout protéiques) provoqués par les changements métaboliques chez ces femelles et par l'augmentation des besoins nutritionnels liées au parasitisme (Houdijk, 2012 ; Houdijk et al., 2012 ; Perri et al., 2011).

✓ Effet de la parité (multi vs primipares):

Certaines études ont montré des niveaux d'OPG divergents en fonction de la parité chez les ovins (Haile et al., 2007) et les caprins (Hoste et al., 1999 ; Morris et al., 1997). Néanmoins, Etter et al. (2000) n'ont pas observé de différences d'OPG entre les primipares et les multipares. Cet effet de la parité serait en fait étroitement liée à l'âge et au premier contact avec les nématodes car, en pratique, que ce soit en élevage ovins ou caprins, les jeunes femelles ne sortent pas ou peu avant les premières mise-bas et, ainsi, sont peu en contact avec les L3s.

✓ Niveau de production :

Une réceptivité élevée au parasitisme GI et des conséquences pathologiques plus sévères ont été rapportées à plusieurs reprises chez les animaux présentant les meilleurs niveaux de production (Chartier et Hoste, 1994 ; Etter et al., 2000 ; Hoste et al., 1999 ; Veneziano et al., 2007). Chez ces animaux les plus performants, ces niveaux d'infestation plus élevés pourraient s'expliquer par à une plus forte consommation d'herbe au pâturage conduisant à des infestations plus conséquentes, en raison d'ingestion de L3 plus élevée. Néanmoins, comme pour le phénomène de « periparturient rise » évoquée précedemment, l'hypothèse de déséquilibres alimentaires exacerbés, liés à des besoins nutritionnels plus élevés, a également été envisagée et en partie démontrée par des expériences de supplémentations ajustées (Falzon et al., 2013 ; Kidane et al., 2010).

1.4. Conséquences des strongyloses gastro-intestinales chez les ruminants

En général, chez les petits ruminants, les strongyloses digestives évoluent sous forme chronique, d'expression subclinique, entrainant des déficits de productions.

1.4.1. Impact sur la production

En terme économique, chez les ruminants, les strongyloses GIs sont reconnues mondialement, comme une des premières pathologies en raison des pertes de production majeures qu'elles entrainent. En élevage, la présence des vers influence à la fois la quantité et la qualité des productions (Charlier et al., 2014). De multiples études ont montré que le parasitisme GI est responsable de retard de croissance des jeunes animaux (Urquhart et al., 1996) que ce soit chez les agneaux (Kyriazakis et al., 1996) ou les chevreaux (Torres-Acosta, 1999) qui se traduisent par des réductions de poids de carcasse à l'abattage. Par ailleurs, des modifications de la qualité des carcasses ou de la viande (taux de gras réduit; rétention d'eau) ont été décrites à la suite d'infestations par les nématodes GIs (Sykes et Coop, 1976, 1977). Chez les femelles laitières, les infestations par les trichostrongles digestifs ont été régulièrement associées à des baisses de production de lait (Chartier et Hoste, 1994 ; Veneziano et al., 2007) et, de manière plus rare, à des modifications de la composition du lait (Chartier et Hoste, 1994 ; Hoste et Chartier, 1993), chez les plus fortes productrices. Enfin, de multiples exemples en Australie et en Nouvelle-Zélande ont illustré les baisses de production et les altérations de la qualité de la laine, chez les animaux parasités (Knox et al., 2006).

1.4.2. Tableau clinique des strongyloses gastro-intestinales

L'évolution clinique des strongyloses digestives est généralement chronique. L'état des animaux évolue peu à peu et peut conduire à la mort. D'abord, des symptômes généraux apparaissent tels une baisse d'appétit, un amaigrissement progressif, une asthénie ou des signes de malnutrition (Urquhart et al., 1996). Les signes de gastroentérites accompagnées de diarrhées aiguës sont également très fréquents (Williams et al., 2010). De plus, des symptômes plus spécifiques peuvent aussi être constatés en fonction des espèces en cause (Urquhart et al., 1996). Ainsi, dans le cas d'infestation par des nématodes hématophages, comme *H. contortus*, des signes d'anémie et des œdèmes sous maxillaires ou en partie déclives sont fréquemment décrits (Jacquiet, 1997 ; Urquhart et al., 1996). Il a été estimé qu'un mouton parasité par 5000 *H. contortus* perdait l'équivalent de 250mL de sang par jour (Urquhart et al., 1996).

1.4.3. Mécanismes physiopathologiques

Les baisses de production et les symptômes observés chez les animaux parasités s'expliquent par des perturbations de la physiologie digestive. Les principaux processus physiopathologiques impliqués ont été largement décrits. A l'inverse, les mécanismes pathogéniques à la base de ces perturbations restent mal identifiés.

- ✓ Diminution de l'ingestion :

Les infestations par les nématodes GIs s'accompagnent généralement d'une baisse d'appétit. Dans le cas d'infestation massive, cette perte d'appétit peut aller jusqu'à une anorexie quasi totale (Knox et al., 2006 ; Urquhart et al., 1996).

- ✓ Mal-digestion et malabsorption :

Les nématodes présents dans les différents organes digestifs provoquent d'importantes lésions des divers épithéliums. Dans l'abomasum, la présence des vers est associée à des modifications des glandes gastriques et à une réduction de la densité de cellules différenciées, en particulier les cellules à HCl. Dans l'intestin, une abrasion des villosités et une altération sévère des entérocytes sont les principales lésions décrites (Hoste et al., 1997).

Ces modifications structurales ont d'importantes répercussions fonctionnelles, en particulier des conséquences sur la digestion des aliments et l'absorption des nutriments dans les différents organes digestifs. Le parasitisme GI induit une augmentation du pH gastrique entrainant une moindre efficacité de la pepsine et une profonde déplétion des activités enzymatiques intestinales. De plus, il est également à l'origine d'une l'altération de la perméabilité des épithéliums (Hoste et al., 1997). Enfin, des perturbations du péristaltisme réduisant le temps de contact du chyme avec les muqueuses ont aussi été signalées (Hoste et al., 1997). La conjonction de ces phénomènes affectant les structures et les fonctions digestives est à l'origine d'une mal-digestion des aliments et d'une malabsorption des nutriments (Knox et al., 2006; Williams et al., 2010).

- ✓ Altérations des métabolismes :

Les effets du parasitisme GI sur l'appétit et la digestion sont amplifiés par une réorientation des métabolismes (Hoste et al., 1997). Le résultat global des réductions d'ingestion et d'absorption est une diminution des apports nutritionnels. En parallèle, la présence des vers augmente les besoins nutritionnels de l'hôte pour maintenir l'homéostasie sanguine et l'intégrité des épithéliums digestifs et développer une réponse immunitaire (Coop et Kyriazakis, 2001; Kyriazakis et Houdijk, 2006). Il en résulte une réquisition des nutriments, en particulier les protéines, vers les sites endommagés par les vers au détriment des sites habituels de synthèse (mamelle, follicule pileux, muscle), ce qui accroît les pertes de production. Une forte perturbation du métabolisme protéique découle alors du parasitisme par les nématodes GIs (Hoste et al., 2005b). De même, Knox et al. (2006) ont suggéré que les métabolismes du phosphore, du calcium et du fer sont modifiés par la présence des vers.

1.4.4. Mécanismes pathogéniques

- ✓ Effets mécaniques :

Les lésions des muqueuses digestives sont dues en partie à un effet mécanique des nématodes lors de la fixation des nématodes aux épithéliums, les vers pouvant dissocier ou endommager les tissus digestifs de l'hôte en raison de structures anatomiques spécialisées (Hoste et al., 1997). Certaines espèces des Strongylidae (*Chabertia ovina* par exemple) présentent une capsule buccale développée équipée de dents qui leur permettraient de se fixer aux épithéliums digestifs (Urquhart et al., 1996). Chez les Trichostrongyloidae, la capsule buccale est réduite et seule l'espèce hématophage *H. contortus* présente une néoformation dentale. Pour les espèces intestinales, comme *Trichostrongylus* spp., un effet abrasif de la cuticule du vers sur les entérocytes a été évoqué (Hoste et al., 1997).

- ✓ Effets des produits d'excrétion-sécrétion :

La plupart des nématodes GIs libèrent dans leur environnement des produits d'excrétion-sécrétion (E/S) de nature biochimique diverse, particulièrement des protéines qui en général présentent une activité enzymatique (protéase, acétylcholinestérases)

(Hoste et al., 1997 ; Mallet et Lesage, 1987). Le rôle exact de ces produits E/S reste mal connu, mais leur intervention dans l'installation des L3s, le développement, la nutrition et la reproduction des vers chez l'hôte est suspecté. Certaines des molécules libérées sont ainsi toxiques pour les cellules de l'hôte et contribueraient à la genèse des perturbations physiopathologiques (Hoste et al., 1997).

L'importance économique et sanitaire des strongyloses gastro-intestinales conduit à rechercher la maitrise de ces infestations à des niveaux acceptables avec les objectifs de productions des élevages. Pour ce faire, les molécules anthelminthiques de synthèse ont pendant plus de 50 ans représentaient des solutions idéales en raison de leur facilité d'emploi et de leur coût abordable. Toutefois, ces traitements anthelminthiques rencontrent désormais de plus en plus de limites, notamment en élevage des petits ruminants. En conséquence, une approche plus intégrée de la gestion de ce parasitisme digestif est désormais privilégiée. Elle vise à associer plusieurs solutions alternatives ou complémentaires aux AHs de synthèse (sans d'ailleurs les exclure) afin d'assurer une maitrise plus durable des infestations (Hoste et Torres-Acosta, 2011). Parmi ces approches, l'exploitation comme nutricaments (Hoste et al., 2015) de ressources, notamment fourragères, contenant des tanins est au coeur de cette thèse.

2. Les anthelminthiques de synthèse et leurs limites

La première molécule anthelminthique (AH) de synthèse (appelée pipérazine) avec d'effet sur les nématodes, a été développée en 1953. Depuis cette date, la maitrise des strongyloses GIs des ruminants repose prioritairement sur l'administration répétée de molécules anthelminthiques (AHs) de synthèse (Kaplan, 2004). L'AH idéal est un traitement multivalent (contre diverses espèces mais aussi divers stades parasitaires), non-toxique et rapidement éliminé par l'hôte, facile d'administration et d'un coût raisonnable. Pendant de nombreuses années, les AHs de synthèse se sont avérés efficaces. Cependant, l'utilisation de ces molécules rencontre de plus en plus de limites, à cause des résistances.

2.1. Classification des anthelminthiques de synthèse

Les AHs de synthèse se répartissent en quatre familles à large spectre selon leur mode d'action : les benzimidazoles et les pro-benzimidazoles, les imidazothiazoles et les lactones macrocycliques (Tableau 2). Par ailleurs, en 2008 une nouvelle molécule a été lancé (le monepantel) qui représente le précurseur d'une quatrième famille (dérivés de l'amino-acétonitrile = AAD) (Kaminsky et al., 2008, 2009).

Les benzimidazoles et les pro-benzimidazoles sont efficaces contre les strongles GIs et respiratoires. Certaines molécules de cette famille présentent également une activité contre les douves et les tenias. Les imidazothiazoles et les tétrahydropyrimidines sont actifs contre les vers GIs et pulmonaires. Les lactones macrocycliques sont également appelées endectocides car elles sont actives à la fois contre les nématodes GIs mais aussi certains ectoparasites (acariens ou insectes) (Bengone-Ndong et Alvinerie, 2004 ; Urquhart et al., 1996).

Enfin, certaines molécules AHs à spectre étroit, tel le closantel, dirigé contre *H. contortus* et d'autres parasites hématophages, sont également utilisées en médecine vétérinaire, mais de façon beaucoup moins large que les familles précedentes.

Tableau 2. Principales molécules à activité AH contre les strongles chez les petits ruminants

Familles	Représentants	Spectre d'activité	Cibles suspectée chez les vers
Benzimidazoles et Pro-Benzimidazoles	Albendazole Oxfendazole Febantel Fenbendazole Thiabendazole Mebendazole Netobimin	SGI SGI/SR SGI/SR SGI/SR SGI SGI SGI/SR/douve	β-Tubuline
Imidazothiazoles et Tetrahydropyrimidines	Levamisole Pyrantel, Morantel	SGI/SR	Récepteurs à l'acetylcholine
Lactones Macrocycliques	Doramectine Eprinomectine Ivermectine Moxidectine Abamectine	SGI/SR, Insectes, Acariens	Canaux ioniques Récepteurs GABA
Dérivés d'Amino-acétonitriles (AAD)	Monepantel	SGI/SR	Agoniste nicotinique

SGI= strongles gastro-intestinaux ; SR= strongles respiratoires

2.1.1. Les benzimidazoles et les pro-benzimidazoles

Cette famille regroupe de nombreux représentants qui présentent une structure chimique commune. Ces molécules sont administrées par voie orale uniquement. Ils sont commercialisés sous forme de solution, de bolus ou incorporés dans des blocs de compléments minéralo-vitaminiques (Smith et Sherman, 2009). Les benzimidazoles agissent directement sur les vers alors que les probenzimidazoles sont administrés à l'animal sous forme de prodrogues converties en molécules actives par des réactions enzymatiques, qui se déroulent en général dans le foie (Lanusse et Prichard, 1993). Les représentants de cette famille ont un mode d'action commun : ils se fixent spécifiquement aux molécules de β-tubuline des vers (Samsom-Himmelsjerna, 2007a). Par cette fixation, ils empêchent la synthèse et la polymérisation des microtubules dans les cellules tégumentaires et intestinales des nématodes, sans altérer le réseau de microtubules de l'hôte. Par ailleurs, les benzimidazoles perturbent des fonctions essentielles telles le maintien de la morphologie cellulaire, le mouvement des organites et la mitose, ce qui conduit à la mort du vers.

2.1.2. Les imidazothiazoles et les tétrahydropyrimidines

Les imidazothiazoles (lévamisole) et les tétrahydropyrimidines (pyrantel, morantel) ont des structures chimiques différentes, mais partagent le même mode d'action. Pour cette raison, ils sont regroupés dans une même famille (Tableau 2). Ces AHs se fixent sur les récepteurs nicotiniques à acétylcholine des nématodes GIs (Samsom-Himmelsjerna, 2007a). Ils miment l'action de l'acétylcholine. Cette fixation induit un changement de la perméabilité membranaire post-synaptique provoquant une contraction musculaire, suivi d'une paralysie spastique et finalement, la mort des vers.

2.1.3. Les lactones macrocycliques

Les lactones macrocycliques représentent une classe homogène en raison du mode d'action unique et spécifique (Bengone-Ndong et Alvinerie, 2004). Cette famille regroupe les avermectines (ivermectine, doramectine, eprinomectine) et les mylbémycines (moxidectine) (Tableau 2) (Beugnet et al., 1997). Ces molécules ont une structure chimique complexe avec de nombreux hétérocycles lactones. Leur mode d'action reste encore mal élucidé, mais plusieurs hypothèses sont évoquées. Il semble que les lactones macrocycliques se fixent aux canaux ioniques glutamate-dépendant de la membrane des cellules neuromusculaires des nématodes et des arthropodes (Samsom-Himmelsjerna, 2007a). Cette fixation provoquerait une augmentation de la perméabilité aux ions chlorures (Bengone-Ndong et Alvinerie, 2004 ; Beugnet et al., 1997 ; Urquhart et al., 1996). Ceci entraînerait une inhibition du contrôle nerveux des muscles du pharynx, de l'utérus et des muscles du corps du vers, conduisant à la mort du vers par paralysie. Un mécanisme additionnel des lactones macrocycliques, a aussi été mentionné l'antagonisme vis- à- vis de l'acide gamma amino butyrique (« GABA », selon les initiales en anglais) (Sumano et Ocampo, 1997).

2.1.4. Le monepantel

Depuis le lancement des lactones macrocycliques au début des années 1980, aucune nouvelle famille de molécule AH n'avait été lancée jusqu'en 2008: le monepantel, une molécule appartenant à la classe des dérivés d'amino-acetonitrile (AAD) a alors été commercialisée (Kaminsky et al., 2008).

Concernant le mode d'action du monepantel, des études suggèrent qu'il agirait comme un agoniste nicotinique, pour un récepteur nicotinique lie à une acétylcholinestérase, spécifique (Kaminsky et al., 2008). Le spectre d'activité du monépantel inclut les stades 4 larvaires et les stades préadultes et adultes d'un large spectre d'espèces de nématodes. De plus, le monépantel serait efficace contre les souches résistantes aux trois autres familles d'anthelminthiques à large spectre. Ces résultats d'efficacité sont complétés par la bonne tolérance et une faible toxicité pour les mammifères. L'utilisation proposée chez les ruminants est celle d'une administration unique par voie orale (2,5 mg/kg, 3,5 mg/kg de poids vif ou 5 mg/kg de poids vif, chez les ovins, caprins et les bovins, respectivement) (Kaminsky et al., 2008, 2009 ; Rolfe et al., 2009 ; Sager et al., 2009).

Néanmoins, pour cette molécule récente, la présence de souches résistantes de *T. circumcinta* a d'ores et déjà été rapportée, tout d'abord en Nouvelle-Zélande puis en Australie (Leathwick et al., 2013 ; Scott et al., 2013). Plus récemment des souches résistantes au monépantel d'*H. contortus* ont aussi été décrites aux Pays Bas (Van den Brom et al., 2015) et au Brésil (Costa-Junior et al., 2015).

2.2. Les limites d'utilisation des anthelminthiques de synthèse

L'utilisation des AHs de synthèse dans la maîtrise des strongyloses GIs est désormais confrontées à plusieurs limites, liées notamment à la présence de résidus dans l'environnement ou dans les produits de consommation et à la pression sociétale. Cependant, c'est surtout le développement mondial de résistances aux AHs de synthèse dans les populations de nématodes GIs qui est le phénomène le plus préoccupant, en particulier chez les petits ruminants (Jackson et al., 2012).

2.2.1. Ecotoxicité des anthelminthiques

Les AHs de synthèse sont généralement métabolisés dans le tractus digestif de l'animal ou par le foie après absorption (Mc Kellar, 1997). La plupart des molécules AHs sont ainsi retrouvées dans les matières fécales, en quantité plus ou moins importante, sous la forme active ou de métabolites. Depuis environ deux décennies, des études se sont focalisés sur l'activité de ces AHs ou de leurs métabolites sur le fonctionnement de l'écosystème de la prairie et sur leurs possibles conséquences pour certains composants biotiques (Beynon, 2012). En particulier, certaines lactones macrocycliques présenteraient une toxicité pour des insectes coprophages (Mc Kellar, 1997). Cette écotoxicité potentielle s'explique par le spectre d'action (incluant les insectes) des endectocides, mais elle semble surtout liée à certains modes d'administration (bolus) entrainant une persistance des molécules et des résidus dans les matières fécales (Beynon, 2012).

2.2.2. Restrictions d'emploi des anthelminthiques

Actuellement, les préoccupations des consommateurs des pays développés vont en augmentant sur la qualité des produits d'origine animale, pour réduire l'emploi d'intrants chimiques en élevages et éviter la présence de résidus chimiques dans les produits d'origine animale. En conséquence, les règles d'utilisation des médicaments de synthèse, incluant les AHs, sont de plus en plus contraignantes, pour limiter leur usage dans certaines productions. Ces restrictions sont particulièrement fortes chez les ruminants laitiers et dans les modes de production répondant aux critères de l'^Agriculture Biologique'.

2.3. Résistance des nématodes gastro-intestinaux aux anthelminthiques

Une population de nématodes GIs résistante aux AHs se définit comme une population ayant génétiquement acquis la capacité de résister à des concentrations d'AHs habituellement létales pour des individus de cette espèce (Jackson et al., 2012). La résistance repose sur un mécanisme génétique et se transmet donc de manière héréditaire (Coles, 2002 ; Wolstenholme et al., 2004) mais le phénomène de résistance aux AH est évolutif. Ainsi, les parasites devenus résistants par mutation génétique sont au début peu nombreux, mais leur développement et leur abondance sont favorisés par une pression de sélection, lié à l'emploi répété d'anthelminthiques (Waller, 2006a, 2006b).

La première résistance aux AH décrite a concerné la pipérazine, dix ans après son lancement. Ensuite, la première famille d'AH à large spectre (les benzimidazoles) a aussi été concernée très tôt par le développement puis l'expansion de résistances (Jackson et al., 2012 ; Kaplan, 2004). Globalement, pour chaque nouvelle molécule qui est mise sur le marché, les nématodes GI ont développé une résistance dans un intervalle de temps d'environ 10 ans (Kaplan, 2004 ; Waller, 2006a). Aujourd'hui, toutes les classes d'AHs de synthèse sont concernées par ce phenomène (Bartley et al., 2015; Van der Brom et al., 2015 ; Wolstenholme et al., 2004) qui concerne tous les continents (Jackson et al., 2012 ; Kaplan, 2004 ; Sutherland et Leathwick, 2011). De plus, des cas de résistances multiples ont été détectés dès les années 1980s (Chandrawathani et al., 1999 ; Kaplan, 2004 ; Yue et al., 2003) et continuent de se répandre.

Les cas de résistance aux AHs ont surtout été décrits chez les petits ruminants et la prévalence des résistances est plus importante chez les caprins que chez les ovins (Chandrawathani et al., 1999 ; Jackson et al., 2012). Pendant longtemps, les cas de résistance ont été absents chez les bovins, cependant, ils sont de plus en plus fréquemment signalés (Cotter et al., 2015 ; Kaplan, 2004 ; Martinez-Valladares et al., 2015).

Des résistances aux AHs ont désormais été mentionnées pour toutes les espèces de nématodes GIs des petits ruminants, principalement pour *H. contortus*, *Trichostrongylus* spp. et *Teladorsagia* spp. (Besier, 2007 ; Kaplan, 2004). Chez les bovins, elles ont été rapportées pour: *Cooperia* spp., *Ostertagia* spp., et *Oesophagostomum* spp. (Sutherland et Leathwick, 2011).

2.3.1. Mécanismes des résistances aux anthelminthiques

La résistance aux AHs peut s'expliquer par 3 phénomènes : 1) la modification de la molécule cible; 2) le changement des voies métaboliques qui conduirait à l'inactivation ou à l'élimination accélérée des molécules AHs; 3) l'amplification du nombre de cibles pour diluer l'activité des molécules AHs chez le parasite (Wolstenholme et al., 2004). La résistance initiale chez certains vers de la population serait préacquise à la suite de mutation génétique(s). Les mécanismes de résistance seraient spécifiques pour chaque famille de molécules AHs (Samsom-Himmelsjerna, 2007b).

Les mécanismes de résistance aux divers familles des AH qui ont été identifiées, peuvent se résumer comme suit. Pour les Benzimidazoles, les plus largement étudiés, le développement de résistance serait dû à une mutation du gène codant la β-tubuline (Samsom-Himmelsjerna, 2007b ; Wolstenholme et al., 2004). Pour le lévamisole, la résistance serait associée à des modifications des récepteurs nicotiniques (Samsom-Himmelsjerna, 2007b ; Sansger et Gill, 1999). Pour les lactones macrocycliques, la résistance serait un phénomène plus complexe car reposant sur des mécanismes de mutation des gènes impliqués dans la réponse cellulaire au GABA ou de surexpression de la glycoprotéine-P impliquée dans la détoxification cellulaire (Samsom-Himmelsjerna, 2007b ; Sangster et Gill, 1999 ; Wolstenholme et al., 2004). Ce dernier mécanisme est aussi suggéré pour expliquer les cas de résistances multiples.

Tout renforcement de la pression de sélection associée à l'emploi de AHs favorise l'apparition de résistances (Wolstenholme et al., 2004), car à la suite de traitements répétés, les nématodes GIs porteurs d'allèles 'résistants', notamment les homozygotes, survivent contrairement à leurs congénères sensibles. En conséquence, la proportion d'allèles 'résistants' augmente dans la population. Les vers résistants peuvent ensuite se reproduire et propager les allèles de résistance. Différents facteurs, dépendant des nématodes (intrinsèques) ou liés à l'activité humaine (extrinsèques), peuvent accélérer l'apparition et à la propagation de la résistance au sein d'une population de vers.

2.4. Gérer l'apparition des résistances aux anthelminthiques.

Pour préserver l'efficacité des traitements AHs et ralentir l'apparition et la diffusion des résistances chez les vers, il est apparu nécessaire d'utiliser ces molécules de façon beaucoup plus raisonnée, à partir de la compréhension des facteurs favorisant le développement des résistances aux AHs (Kotze et al., 2014 ; Leathwick et Besier, 2014 ; Nabukenya et al., 2014).

Cette prévention des résistances repose sur des solutions prenant le contrepied des erreurs de conduites dans l'application des traitements identifiées comme créant des pressions de sélection trop forte (traitements systématiques répétés trop fréquemment, ou appliqués à l'ensemble des animaux d'un troupeau. L'idée principale est désormais de traiter de façon plus pertinente soit

- en mieux identifiant les périodes à fort risque parasitaire (« Targeted Treatment » = TT) (Kenyon et Jackson, 2012).

- ou en identifiant les animaux les plus infestés représentant un risque majeur de contamination du pâturage et donc un risque pour les congénères du troupeau. Dans ce cas, le but est de n'administrer les traitements qu'à ces seuls animaux. Ce traitement ciblé est décrit en Anglais sous le terme « Targeted Selective Treatment » (acronyme TST). Cette application raisonnée ciblant une proportion des animaux du troupeau peut s'appuyer sur divers types de critères de repérage des animaux les plus parasités: sub clinique (comme dans le cas de la méthode FAMACHA) (Van Wyk et Bath, 2002; Van Wyk et al., 2006) ou pertes de productions zootechniques appliqués sur des agneaux en croissance (Charlier et al., 2014 ; Kenyon et Jackson, 2012) ou en fonction de la production de lait chez des chèvres laitières (Hoste et al., 2002a, 2002b). Ces méthodes TST dans leur ensemble vise à exploiter le concept de refuge (Van Wyk, 2001) en préservant des gènes de sensibilité aux AHs dans les populations de Nematodes.

3. Alternatives aux anthelminthiques de synthèse

En raison des limites croissantes à l'efficacité des AHs de synthèse, la nécessité de mettre en place des méthodes alternatives pour maîtriser les strongyloses GIs est désormais de plus en plus prise en compte. Ces méthodes reposent sur 3 principes de lutte visant à : **1)** tarir la source de contamination des hôtes, **2)** éliminer les nématodes GIs au sein de l'hôte ou **3)** augmenter la résistance de l'hôte (Hoste et Torres-Acosta, 2011).

Certaines méthodes relevant de ces trois principes ne seront que brièvement présentées. Par contre, l'utilisation de plantes riches en tanins condensés sera plus détaillée. Ce volet des interactions entre «*Nutrition de l'hôte et infestations par des nématodes gastro-intestinaux*» en prenant pour modèle l'espèce *Haemonchus contortus* est aussi largement développée dans la partie bibliographique de cette thèse.

3.1. Tarir la source de contamination des animaux

Tarir la contamination du pâturage a pour but de bloquer le cycle biologique des nématodes GIs en maîtrisant son infestivité, et de réduire ainsi les risques de contact entre hôtes sensibles et L3s (Heckendorn, 2007). Diverses méthodes agronomiques de gestion du pâturage existent pour atteindre cet objectif. Elles reposent sur trois axes (Osoro et al., 2009):

- ✓ La prévention, consiste à mettre des animaux sains sur des pâtures propres, donc exemptes de L3s.
- ✓ L'évasion, consiste à transférer des animaux traités par des AHs de pâtures contaminées vers des pâtures propres.
- ✓ La dilution vise à réduire l'infestivité (le risque parasitaire) du pâturage (Mahieu, 2013).

Les deux premières options (prévention et évasion) supposent la disponibilité de prairies propres ou présentant des niveaux faibles de contamination par les L3. Une première méthode pour y arriver repose sur une mise au repos prolongée des paturages, basée sur une rotation des parcelles. Le but est d'attendre la mort naturelle des L3s des nématodes GIs présentes sur une prairie avant le retour des hôtes sur cette même parcelle (Hoste et al., 2004 ; Legarto et Leclerc, 2007). En pratique, cette méthode est surtout applicable en zones tropicales, en raison de la survie plus courte des L3s dans ces régions

(2 mois) par rapport aux régions tempérées (6 à 12 mois) (Barger, 1999 ; Hoste et al., 2004).

La méthode de dilution d'infestivité du pâturage peut se réaliser par une diminution de chargement (Etter et al., 2000), par le mélange d'animaux sensibles au parasitisme avec des animaux résistants soit entre animaux d'âges différents soit par pâturage mixte, alterné ou simultané, entre 2 espèces animales ayant des spécificités différentes pour des espèces de nématodes GIs (par exemple : bovin/ovin, chevaux/petits ruminants) (Barger, 1999 ; Mahieu, 2013 ; Niezen et al., 1996 ; Rocha et al., 2008 ; Waller, 2004).

Par ailleurs, des méthodes de décontamination active des prairies (chimiques, physiques (application de pratiques culturales), lutte biologique) visent à réduire le niveau des L3s présentes dans l'environnement et constituant un risque pour les ovins et caprins.

L'utilisation de substances chimiques, telles la chaux ou la cyanamide calcique, a été évoquée pour décontaminer les prairies par les L3 (Hoste et al., 2004). Néanmoins, en élevages, leur emploi s'est souvent avéré décevant (Hoste et al., 2004).

Certaines pratiques culturales, comme le retournement par labour des prairies (Hoste et al., 2004) ou le brûlage contrôlé des pâturages (écobuage) (Hounzangbe-Adote, 2004) permettent de décontaminer les prairies. La fauche avant usage est aussi un moyen intéressant pour réduire la contamination des repousses (Legarto et Leclerc, 2007).

Enfin, des méthodes de maitrise fondée sur des agents biologiques ont été considérées. En particulier, l'exploitation de champignons nématophages (c'est-à-dire des champignons qui parasitent les stades libres des nématodes) est une des méthodes de décontamination des pâturages proposées, fondée sur un principe de lutte biologique. En tuant des œufs ou les larves sur la prairie, ces espèces de champignons en réduisent l'infestivité (Cruz et al., 2015 ; Mahl et al., 2012 ; Silva et al., 2010 ; Waller et Thamsborg, 2004). Aujourd'hui, plus de 200 espèces de champignons nématophages ont été décrites. Toutefois, les travaux ont porté, en majorité, sur l'utilisation de spores de l'espèce *Duddingtonia flagrans* pour maîtriser le parasitisme des petits ruminants (Santurio et al., 2011), ou des bovins (Assis et al., 2012). Par rapport aux méthodes chimiques, la lutte biologique a plusieurs avantages: l'absence de toxicité pour l'animal ainsi que l'absence d'effets négatifs sur la dégradation des fèces et sur la faune prairiale (Knox et al., 2002 ; Paraud et al., 2007).

3.2. Améliorer la résistance de l'hôte

3.2.1. La sélection d'animaux génétiquement résistants

La sélection d'animaux résistants aux nématodes GIs est une approche envisagée, sur des bases scientifiques, depuis près de 50 ans afin de réduire l'emploi des AHs des synthèses (Pomroy, 2006), en sélectionnant des animaux avec une réponse immunitaire plus efficace contre les parasites. Ils auront donc des infestations moins intenses chez l'hôte et *in fine* une diminution progressive de la contamination des pâturages (Baker et al., 1998 ; Windon, 1996). La variabilité génétique de résistance aux nématodes GIs chez l'hôte a été signalée soit entre races, soit intra race (entre individus d'une même race) (Andronicos et al., 2010 ; Bishop et Morris, 2007 ; Terefe et al., 2007).

Par contre, la sélection d'animaux résistants peut présenter certains aspects négatifs, soit par le risque d'une augmentation de sensibilité à d'autres agents pathogènes (Gruner et al., 1998) ou en affectant la productivité (Gray, 1997). De plus, ces programmes sont développés à long-terme et doivent prendre en compte les conditions locales d'élevage, et la disponibilité des races présentes pour répondre aux objectifs d'élevage (Pomroy, 2006 ; Windon, 1996).

3.2.2. La vaccination

Le principe général de la vaccination vise à mettre en contact préventivement l'hôte avec de faibles doses d'antigènes d'agent pathogènes (y compris des parasites) de manière à stimuler ses défenses immunitaires et ainsi, à le protéger vis-à-vis de tout contact futur par ces mêmes agents pathogènes (Jackson et Miller, 2006 ; Ketzis et al., 2006 ; Smith et al., 2001 ; Waller et Thamsborg, 2004). Les premiers essais pour utiliser la vaccination contre les nématodes GIS datent des années 1960s par l'utilisation de L3s irradiées d'*H. contortus* et de *T. colubriformis* (Mulligan et al., 1989).

Malgré des résultats prometteurs, la stratégie d'une vaccination contre les nématodes GIs rencontre encore plusieurs limites. Aujourd'hui, il semble que les possibilités d'avoir un vaccin efficace contre les strongyloses reste limité à la seule espèce *H. contortus* (Bassetto et al., 2014a, 2014b ; Molento et al., 2011).

3.2.3. L'amélioration de la ration de l'hôte

Les principes en relation avec la manipulation de la nutrition animale pour améliorer la réponse de l'hôte contre les parasites sera discutée largement dans le chapitre d'etude bibliographique.

En bref, les strongyloses GIs provoquent de sévères perturbations de la physiologie digestive et induisent une augmentation des besoins alimentaires de l'hôte. Donc, un des moyens pour pallier aux fortes perturbations des métabolismes surtout protéique mais aussi énergétique (Hoste et al., 2005b ; Houdijk, 2012; Houdijk et al., 2012 ; Kidane et al., 2009 ; Knox et al., 2006) est de proposer une complémentation nutritionnelle adaptée. En partant de ce constat, il a été suggéré qu'une amélioration de la ration alimentaire permettant de couvrir les besoins supplémentaires associés à la présence des nématodes, contribuerait à améliorer la réponse de l'hôte au parasitisme en particulier lorsque les corrections touchent la principale ressource limitante de la ration (Athanasiadou et al., 2008; Houdijk, 2012).

Le concept d'immunonutrition a été suggéré car l'amélioration de la ration conduit à une meilleure résilience par une réduction des conséquences des infestations subcliniques et à une résistance améliorée (Kidane et al., 2010 ; Kyriazakis and Houdijk, 2006).

3.3. Eliminer les nématodes gastro-intestinaux

Afin de limiter l'émergence de nouveaux cas de résistances aux AHs, la maîtrise des nématodes GIs pourrait combiner des traitements 'classiques' par les AHs de synthèse, utilisés de façon raisonnée, à des traitements 'alternatifs'. Ces méthodes alternatives reposent notamment sur l'administration de nouvelles substances à propriétés AHs telles que les aiguilles d'oxyde de cuivre ou l'exploitation de plantes à propriétés AHs. Comme pour les aspects quantitatifs de manipulations de la nutrition, ces alternatives représentées par les nutricaments riches en tanins seront discutées plus avant dans le chapitre d'etude bibliographique.

4. Les tanins

4.1. Généralités et définition

Le terme de tanins (ou tannins) correspond à toute substances d'origine végétale ou minérale ayant la propriété de transformer la peau fraîche en un matériau imputrescible, le cuir (Bruneton, 1999). Cette propriété de tannage résulte de la création de liaisons entre les molécules de tanins et les fibres de collagène de la peau.

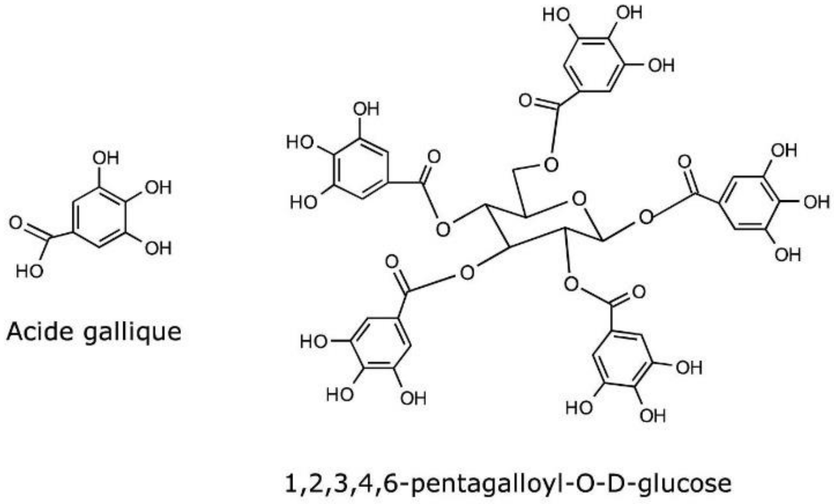
Une première définition chimique pour les tanins a été proposée par Bate-Smith en 1973 : ce sont « des composés phénoliques hydrosolubles ayant un poids moléculaire (PM) compris entre 500 et 3 000 Da qui présentent, à côté des réactions classiques des phénols, la propriété de précipiter les alcaloïdes, la gélatine et d'autres protéines ». Cette définition a été complétée grâce aux méthodes d'analyse récentes qui ont permis d'éclaircir la structure de ces polyphénols. Les tanins sont désormais définis comme des polyphénols de masse moléculaire allant jusqu'à 20 000 Da (Hagerman, 2002). L'extraction des tanins depuis des matières végétales est généralement réalisée par une solution acétone-eau ou méthanol-eau (Makkar, 2000).

4.2. Classification biochimique des tanins

Chimiquement, les tanins sont des polyphénols, et sont considérés comme des métabolites secondaires des végétaux supérieurs (Salminen et al., 2011). La structure des composés phénoliques comprend au moins un noyau aromatique et un ou plusieurs groupements phénoliques. La grande famille des polyphénols s'étend des molécules simples, comme les acides phénoliques, aux molécules hautement polymérisées, comme les tanins. Comme évoqué, il est usuel de distinguer deux groupes de tanins selon leur structure biochimique: les tanins hydrolysables et les tanins condensés (Mueller-Harvey, 2006 ; Salminen et al., 2011).

4.2.1. Les tanins hydrolysables

Les tanins hydrolysables (THs) sont des oligo- ou poly-esters d'un sucre, en général le glucose, et de molécules d'acide-phénol (Salminen et al., 2011). Ils sont classés selon la nature de l'acide-phénol : les tanins galliques possèdent un acide gallique (Figure 2), alors que les tanins éllagiques ont un acide hexahydroxyphénique (Hagerman, 2002). Les tanins éllagiques sont obtenus suite à un couplage oxydatif entre les molécules d'acide gallique (Salminen et al., 2011 ; Salminen et Karonen, 2011).



Acide gallique

1,2,3,4,6-pentagalloyl-O-D-glucose

Figure 2. Les tanins galliques

4.2.2. Les tanins condensés

Les tanins condensés (TCs), ou proanthocyanidols (PAs), sont des polyphénols appartenant à la famille des flavonoïdes (Mueller-Harvey, 2006). Ce sont des oligomères ou polymères des flavan-3-ols, dont la structure chimique des flavonoïdes est basée sur un système d'hétérocycles (Figure 3). En général, les TCs ont des poids moléculaires (PMs) plus élevés que ceux des THs (Mueller-Harvey, 2006).

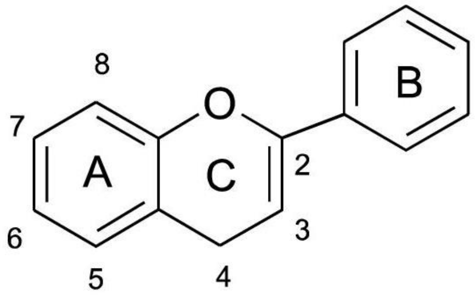


Figure 3. Structure de flavonoïde

L'unité de base (ou monomère) des TCs est un flavan-3-ol (Figure 4). Les TCs sont des polymères de flavan-3-ols liés par des liaisons de type C-C (Type B) ou C-OC (type A)(Mueller-Harvey, 2006). Selon la nomenclature française, les noms des TCs se terminent en «-ols» car ce sont des flavonoïdes (nomenclature anglaise : terminaison en «-in»).

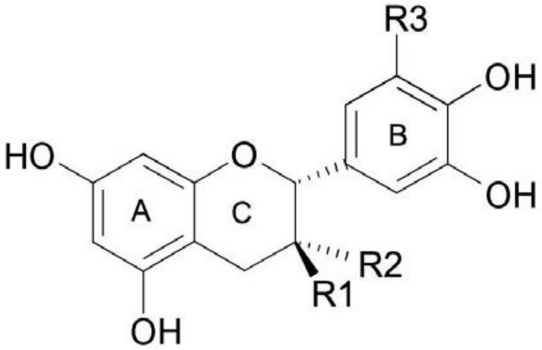


Figure 4. Structure des flavan-3-ols

En fonction du degré de polymérisation (nombre de flavan-3-ols composant le TC), on distingue généralement les oligomères, comprenant de 2 à 10 monomères, des polymères contenant plus de 10 monomères (Mueller-Harvey, 2006).

Les flavonoïdes sont souvent assimilés à des pigments, responsables de la coloration des fleurs, des fruits et parfois des feuilles des végétaux, comme les flavonoïdes jaunes (chalcones, aurones, flavonols jaunes), les anthocyanosides rouges, bleus ou violets. La zone d'absorption de la lumière de la molécule est parfois située dans le proche ultraviolet, une telle « coloration » n'étant alors visible que par les insectes pollinisateurs. De même, certains flavonoïdes participent aussi dans la croissance, le développement et la reproduction des plantes, leur protection contre le rayonnement UV-B, et confèrent des propriétés de défense aux plantes contre divers agresseurs (herbivores) ou pathogènes : insectes, champignons ou bactéries. Selon leur structure de base, les flavonoïdes sont généralement répartis en 6 sous-groupes auxquels il faut ajouter les neoflavonoids, aurons, catéchines (Bruneton, 1999)(Tableau 3).

Tableau 3. Les diverses classes des flavonoïdes

Flavonols	Flavones	Isoflavones	Flavonones	Flavanols	Anthocyanidins
Fisetin	Apigenin	Daidzin	Eriodictyol	Catechin	Apigeninidin
Galangin	Baicalein	Genistein	Hesperidin	Gallocatechin	Cyanidin
Kaempferide	Chrysin	Irilone	Hesperetin	Epicatechin	Delphinidin
Kaempferol	Diosmetin	Luteone	Likvirtin	Epigallocatechin	Diosmetinidin
Morin	Diosmin	Prunetin	Naringin	Epicatechin gallate	Fisetinidin
Myrecitin	Flavonz	Pratensein	Naringenin	Epigallocatechin gallate	Luteolinidin
Quercetin	Luteolin		Picocembrin	Theaflavin	Malvidin
Rhamnetin	Rpoifolin			Theaflavin gallate	Pelargonidin
Robinin	Tangeretin			Theaflavin digallate	Peonidin
Rutin	Techtochrysin			Thearubigins	Robinetidin

4.2.2.1. Structure biochimique des flavan-3-ols

La présence d'un hydrogène (H) ou d'un groupement phénol (OH) aux positions R1, R2 et R3 permet d'identifier les flavan-3-ols (Figure 4).

Les monomères des prodelphinidols (ou prodelphinidins)(PDs) sont différenciés de ceux des procyanidols (ou procyanidins)(PCs) par la présence d'un groupement OH en position R3, ce qui leur confèrent une capacité accrue à se fixer aux protéines (Hagerman, 2012). Parfois, le flavan-3-ol est estérifié avec un acide gallique en position R2 (Figure 5)(Mueller-Harvey, 2006). La présence d'un ou plusieurs acides galliques modifie les propriétés biologiques des TCs (Hagerman, 2012).

Par exemple, le thé vert est connu pour son activité anti-oxydante due à son constituant flavanique majoritaire, le gallate d'épigallocatéchol.

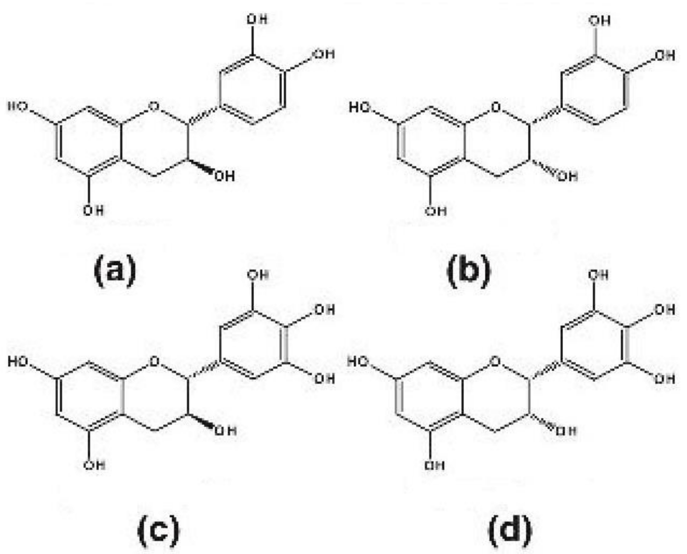


Figure 5. Structure générale des flavan-3-ols et des monomères les plus communs de TCs (Salminen et al., 2011). (a), catechol; (b), epicatechol; (c), gallocatechol; (d), epigallocatechol

Tableau 4. Caractéristiques chimiques des divers Flavan-3-ols

Flavan-3-ols	Classes d'homopolymères	R1	R2	R3	Nombre de fonctions OH
Catechol	Procyanidols	OH	H	H	5
Epicatechol	Procyanidols	H	OH	H	5
Gallocatechol	Prodelphinidols	OH	H	OH	6
Epigallocatechol	Prodelphinidols	H	OH	OH	6
Fisetinididol	Profisetinidols	H	H	H	4
Robinetinidol	Prorobinetinidols	H	H	OH	5

4.2.2.2. Structure biochimique des polymères

Les végétaux supérieurs contiennent majoritairement deux classes d'homopolymère : les PCs composés uniquement de catéchol (C) ou d'épicathécol (EC) ; et les PDs constitués uniquement de gallocathéchol (GC) ou d'épigallocathécol (EGC) (Figure 5) (Bruneton, 1999 ; Mueller-Harvey, 2006). Deux autres classes d'homo-TCs sont plus rarement rencontrées : les profisétidinols (profisetinidins) et les prorobinétinidols (prorobinetinidins) (Mueller-Harvey, 2006). Néanmoins, des hétéro-oligomères, composés à la fois de C ou EC et de GC ou EGC, ont également été identifiés chez certaines plantes, comme le sainfoin (Marais et al., 2000 ; Wang et al. 2015).

Les liaisons interflavaniques sont majoritairement de type Carbone-Carbone (Type B) et surtout établies entre le C8 du monomère terminal et le C6 du monomère additionné. Cependant des liaisons entre le C6 du monomère terminal et le C4 du monomère additionné sont parfois décrites (Hagerman, 2012 ; Mueller-Harvey, 2006). Par conséquent, les TCs peuvent présenter soit une structure linéaire (uniquement des liaisons C4-C8) (exemple des TCs des grains de sorgho), soit une structure branchée (liaisons C4-C8 et C4-C6) (exemple les TCs du quebracho) (Hagerman, 2002) (Figure 6). Un second type de liaison (Type A) a été décrit chez les TCs, qui fait intervenir à la fois une liaison C4-C8 et une liaison C2-O-C7 entre deux monomères (Mueller-Harvey, 2006) (Figure 6).

Le degré de polymérisation des TCs est très variable. Ainsi, en général, dans la nature, on distingue les oligomères de 2 à 10 unités et les polymères de plus de 10 unités (Salminen et Karonen, 2011). Néanmoins, des tanins contenant jusque 83 unités et un poids moléculaire de 23 900 g mol^{-1} (Cheynier et al., 1999) ont été décrits. Toutefois, des TCs de plus haut degré de polymérisation existeraient, mais leur insolubilité rend leur analyse difficile (Mueller-Harvey, 2006).

4.2.2.3. Biosynthèse des tanins condensés

Biogénétiquement, les flavan-3-ols et les TCs sont issus de la voie métabolique du phénylpropanoïde (Weisshaar et Jenkins, 1998), qui conduit également à la synthèse des flavonoïdes (Bruneton, 1999). Néanmoins, bien que la voie de biosynthèse des flavonoïdes soit bien connue, les étapes de condensation et de polymérisation des TCs restent mal définies (Hagerman, 2002).

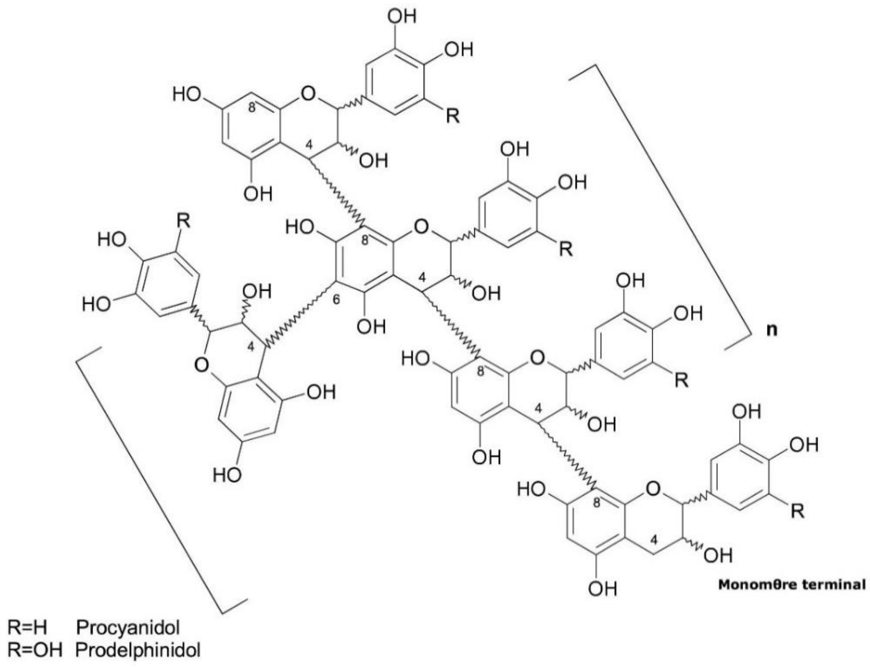


Figure 6. Structure branchée des TCs (Hagerman, 2002)

Les précurseurs de la synthèse des flavonoïdes sont la phénylalanine et l'acétate (Mueller-Harvey et Mc Allan, 1992). Les premières étapes de la synthèse des flavan3-ols seraient communes à celles des anthocyanes et auraient lieu dans le cytoplasme alors que la maturation et la polymérisation des TCs aurait lieu dans la vacuole des cellules végétales (Waghorn, 2008).

4.3. Propriétés des tanins

La structure chimique des tanins présente de nombreux groupements hydroxyles et phénoliques qui leur confèrent la propriété de former des complexes avec de nombreuses macromolécules telles que i) des protéines et ii) des hydrates de carbones, ou encore avec iii) des ions métalliques (Dobreva et al., 2014 ; Patra and Saxena, 2011).

4.3.1. Analyses biochimiques des tanins

En raison de leurs structures complexes et variées, l'analyse des tanins est délicate et leur dosage souvent difficile. Cependant, des méthodes de dosage des tanins totaux (TTs) ont été développées, basées sur les propriétés physicochimiques ou biologiques. Parallèlement, des méthodes ont été proposées pour doser spécifiquement les THs ou les TCs. Récemment, une nouvelle méthode plus efficace et répétable a été développée pour les dosages de TCs (Grabber et al., 2013).

4.4. Mesures des tanins

4.4.1. Méthodes chimiques pour les tanins totaux

La méthode de Folin-Ciocalteu (réactif spécifique des phénols) se décompose en deux dosages colorimétriques successifs : le dosage des phénols totaux puis le dosage des phénols non-tanniques d'un extrait acétonique (Bruneton, 1999 ; Makkar, 2000). Ce second dosage est réalisé après déplétion des tanins de l'extrait par le polyvinyl polypyrrolidone (PVPP) (Makkar, 2000). Le PVPP est une molécule capable de se lier aux tanins présents dans l'extrait et les complexes 'tanin/PVPP' sont retirés de l'extrait par centrifugation (Makkar et al., 1995). Indirectement, la teneur en tanins totaux (TTs) d'un échantillon est déterminée par la différence entre les teneurs en phénols totaux et en phénols non-tanniques.

4.4.2. Méthodes biologiques

Il existe diverses méthodes de dosage des TTs fondées sur leur propriété à se fixer aux protéines :

- ✓ la méthode traditionnelle, utilisant de la poudre de peau, est appliquée dans le cas des drogues officinales (Bruneton, 1999 ; Pharmacopée-Européenne, 2001)
- ✓ la méthode de la diffusion radiale (avec de l'albumine bovine ou du rubesco inclus dans un gel d'agarose) est une méthode simple qui permet le dosage simultané de nombreux échantillons (Hagerman, 2002 ; Makkar, 2000 ; Mueller-Harvey, 2006)
- ✓ les techniques basées sur l'inhibition d'enzyme (!-glucosidase ; trypsine ; alkaline phosphastase) sont des méthodes très sensibles (Schofield et al., 2001)
- ✓ une méthode basée sur la propriété des tanins à réduire la production de gaz lors de fermentations anaérobies a également été développée (Mueller-Harvey, 2006).

4.4.3. Dosages spécifiques et analyses des tanins hydrolysables

Pour le dosage des THs, il est possible d'évaluer la concentration en tanins galliques et éllagiques distinctement. Après hydrolyse sulfurique, l'acide gallique libéré est dosé par la méthode à la rhodamine alors que l'acide éllagique est dosé à l'aide de la méthode à l'acide nitreux (Makkar, 2000 ; Mueller-Harvey, 2001). Ces 2 méthodes colorimétriques peuvent être complétées par une analyse qualitative et quantitative en RMN ou en spectrométrie de masse (Mueller-Harvey, 2001).

4.4.4. Dosages spécifiques et analyses des tanins condensés

Dans la plante, les TCs sont présents sous différentes formes : libres ou liés, c'est-à-dire ceux fixés aux protéines ou aux fibres de la plante (Pérez-Jiménez et Torres, 2011). L'existence de ces deux formes (libres ou liés) rend le dosage des TCs plus délicat.

En général, les TCs sont majoritairement présents sous forme libre (Terrill et al., 1992). Cependant les conditions de stockage, de séchage et d'extraction des échantillons peuvent influencer la proportion de TCs liés/libres (Silanikove et al., 2001). Afin de doser les TCs liés, un traitement par des agents chimiques, tel que le sodium dodécyl sulfate, est réalisé après l'étape d'extraction, afin de rompre les liaisons protéines-TCs et fibres-TCs et de libérer ainsi les TCs liés (Makkar, 2000).

Le dosage des TCs peut être réalisé par diverses méthodes colorimétriques:

- ✓ la méthode à la vanilline, est surtout utilisée dans les études en nutrition (Makkar, 2000). Cependant cette réaction n'est pas spécifique aux TCs mais, de manière générale aux flavanols (Hagerman, 2002).
- ✓ la méthode au Butanol-HCl, développée par Porter et al. (1986), basée sur la réaction de dépolymérisation des TCs en milieu acide. Cette réaction conduit à la libération des anthocyanidines (molécules colorées) correspondants aux monomères clivés (Makkar, 2000). Elle permet un dosage semi-quantitatif des TCs car les monomères terminaux libérés ne donnent pas les anthocyanidines correspondants et par conséquent, ils ne sont pas dosés. La méthode au Butanol-HCl reste la méthode la plus utilisée (Makkar, 2000 ; Mueller-Harvey, 2006).
- ✓ la méthode de l'acétone-HCl-butanol (Grabber et al., 2013) avec addition d'acétone et de l'ion ferrique. Par rapport à la méthode classique, elle a permis d'augmenter la récupération polyphénolique par 1,9 – 3,2 fois plus de tanins depuis les matériaux végétaux. Cette méthode est donc plus sensible, répétable et efficace.

Finalement, l'analyse qualitative et quantitative des TCs d'un échantillon peut être réalisée par thiolyse, une méthode basée sur le clivage des liaisons interflavanique en présence d'un nucléophile (par exemple : le toluène-Į-thiol, ou benzyl-mercaptan) (Mueller-Harvey, 2006). Lorsqu'elle est couplée à une HPLC, cette méthode permet de déterminer le degré de polymérisation moyen des TCs, la nature des monomères terminaux et la proportion de flavan-3-ols de PDs et de PCs (Gea et al., 2011). Enfin, l'étude des TCs d'un extrait ou d'une fraction peut être complétée par des analyses en spectrométrie de masse ou en RMN (Baxter et al., 1997 ; Simon et al., 2003). Cependant, ces analyses sont réservées aux études analytiques approfondies en nutrition.

Lors d'études en parasitologie, l'analyse des échantillons est généralement réalisée par le dosage des TTs et des TCs, en associant des méthodes chimiques (par exemple, Butanol-HCl et Folin-Ciocalteu) et des méthodes biologiques (diffusion radiale).

4.5. Les plantes à tanins

4.5.1. Les plantes à tanins hydrolysables

Ces types des tanins, sont présents chez certaines familles d'Angiospermes dicotylédones et quelques familles de Fagacea, d'Anacardiacea, d'Anacardium et de Geraniaceae (Bruneton, 1999). Par contre, ils sont absents des Gymnospermes et des Monocotylédones (Jean-Blain, 1998).

4.5.2. Les plantes à tanins condensés

Les TCs se rencontrent chez de nombreuses plantes vasculaires chez les Angiospermes et les Gymnospermes. Généralement, les TCs sont plus répandus dans le règne végétal et plus abondants dans les plantes que les THs (Bruneton, 1999 ; Jean-Blain, 1998). Certaines espèces de Fagaceae (chêne et chataîgnier), Pinaceae (pin), Rosaceae (pommier, fraisier) et de Rosidae (acacia) contiennent de fortes quantités de TCs (> 5% de la MS). Parmi les Fabaceae ou Leguminoceae (légumineuses), certaines espèces de légumineuses fourragères, telles que le sainfoin (*Onobrychis viciifolia*), le sulla (*Hedysarium coronarium*) et les lotiers pédonculé et corniculé (*Lotus pedunculatus* et *L. corniculatus*), contiennent des TCs en quantité non négligeable (2 à 5% de la MS).

Par ailleurs, les plantes herbacées de la famille des Poaceae, tel que le ray-grass anglais (*Lolium perenne* L.), ou d'autres représentants de la famille des légumineuses, comme la luzerne cultivée (*Medicago sativa* L.) et le trèfle blanc [(*Trifolium repens* L.) sauf les fleurs], présentent des quantités très faibles ou non détectables de TCs (Carlsen and Fomsgaard, 2008 ; Ramírez-Restrepo et al., 2004).

4.6. Rôle des tanins dans les plantes

Comme évoqué précédemment, les tanins sont des métabolites secondaires des végétaux (Salminen et al., 2011) c'est-à-dire des substances non-essentielles pour la croissance de la plante. Néanmoins, les tanins jouent un rôle dans la défense des plantes face aux agressions. La synthèse des tanins est l'un des mécanismes de défense des plantes contre les attaques des phytopathogènes mais aussi des agresseurs, tels les insectes et les mammifères herbivores (Mueller-Harvey, 2006 ; Salminen et al., 2011). Il a été montré que l'ingestion de tanins affectait l'intégrité du tube digestif des insectes phytophages (Ayres et al., 1997). Par ailleurs, la présence des tanins rend les plantes moins appétentes pour les mammifères herbivores à cause de la sensation d'astringence suite à leur consommation. Cette astringence conduit alors à un arrêt de la consommation et protège ainsi les végétaux d'une prédation excessive (Cala et al., 2012).

4.7. Facteurs de variation de la teneur en tanins

La teneur en tanins d'une plante varie en fonction de plusieurs facteurs intrinsèques, tels l'espèce et la variété, la partie ou le stade végétal, et de facteurs extrinsèques, comme les conditions climatiques, pédologiques ou les stress de prédation (Jean-Blain, 1998 ; Mueller-Harvey et Mc Allan, 1992 ; Waterman, 1999).

4.7.1. L'espèce végétale et la variété

Au sein d'une même famille botanique, les espèces végétales présentent des différences de teneur et de nature des tanins (George et al., 2012). Entre les légumineuses la concentration en TCs diffère largement (McAllister et al., 2005). Ainsi, par exemple : 0,3g TCs/Kg de la MS dans le trèfle, 30g TCs/Kg MS dans le sainfoin (Koupai-Abyazani et al, 1993 ; Marais et al., 2000), 35g TCs/Kg MS dans le sulla (Hoskin et al., 2000). A l'intérieur d'un même genre, il existe aussi des différences entre espèces. Par exemple les teneurs en TCs différent entre le lotier pédonculé (*L. pedunculatus*) (77g TCs/Kg MS) (Foo et al., 1997) et le lotier corniculé (*L. corniculatus*) (48 g TCs/Kg MS) (Foo et al., 1996). Des variations de concentration en TCs ont été observées aussi entre les diverses variétés d'une même espèce de lotier corniculé (*L. corniculatus*) (Hedqvist et al., 2000), de sulla (*H. coronarium*) (Piluzza et al., 2000), et de sainfoin (*O. viciifloia*) (Azuhnwi et al., 2013).

4.7.2. La partie végétale

Les différents organes de la plante présentent des teneurs en tanins variables. Généralement, les plus fortes concentrations sont trouvées dans les fruits, les fleurs et les feuilles, et très peu dans les tiges. Par exemple, il a été mesuré que les feuilles, les fleurs et les tiges de sainfoin contiennent respectivement 0,31%, 0,30% et 0,07% de tanins (Borreani et al., 2003). Aussi, le *Trifolium repens* qui normalement a été considéré comme une fourrage libre de tanins, a une forte concentration de TCS au niveau de ses fleurs (Carlsen et Fomsgaard, 2008).

4.7.3. Le stade végétal

Le stade végétatif influence également la teneur en tanins (Jean-Blain, 1998). Généralement, lors de la croissance de l'appareil végétatif, il y a une dilution des tanins. Dans les feuilles, la quantité et la qualité des tanins changent lors de la maturation. De même, la teneur en tanins diminue généralement lors du mûrissement des fruits. Par exemple, la maturation des feuilles de chêne entre avril et septembre est accompagnée par des modifications de la teneur et une augmentation du degré de polymérisation des tanins (Makkar et al., 1991). Un processus similaire a été observé chez les légumineuses. Ainsi, la teneur en TCs du sainfoin varie de 27 à 16g/Kg de la MS pendant la phase de croissance (Borreani et al., 2003).

La qualité des TCs varie aussi en fonction du stade végétatif (Marais et al., 2000). Lors de la maturation des feuilles de sainfoin, le degré de polymérisation a tendance à augmenter (de 5 à 8,5) de même que le pourcentage de prodelphinidols (de 60 à 95%) (Wang et al., 2015).

4.7.4. Les conditions environnementales

La synthèse des tanins est généralement augmentée en réponse à un stress environnemental quel que soit son origine, tel qu'un stress hydrique, un appauvrissement du sol ou un ensoleillement trop fort (Bennick, 2002). De plus, la proportion de TCs sous forme libre ou liée aux fibres ou aux protéines est également sous l'influence des conditions climatiques et de stress nutritif (Frutos et al., 2002). Ainsi, après une longue période de sécheresse, Feucht et al., (1997) ont observé une augmentation de 7,4 fois de la teneur en flavan-3-ols dans des feuilles jaunissantes par rapport aux feuilles vertes. Récemment, une étude sur les grains de raisin (*Vitis vinifera* L.) a aussi montré que la teneur en flavan-3-ols et la distribution des TCs dans les tissus dépendaient de l'exposition à la lumière et des conditions climatiques (Cadot et al., 2006). La nature du sol affecterait aussi la qualité des TCs puisque les grains de raisins cultivés sur un sol sableux avec peu de réserve en eau ont présenté une proportion de PDs plus faible que ceux cultivés sur un sol plus riche (Cadot et al., 2006). Enfin, un stress engendré par l'agression des végétaux par des herbivores ou des pathogènes induit une synthèse accrue de métabolites secondaires et un stockage important de tanins, en particulier des TCs, au niveau de la zone attaquée (Woodward et Coppock, 1995).

5. Effets des tanins condensés sur les infestations par les nématodes gastro-intestinaux des ruminants.

Cette partie a fait l'objet d'une synthèse, à laquelle je suis associée, et qui a été récemment soumise à « *Advances in Parasitology* » (*Adv. Parasit*.). Cette synthèse porte sur les interactions nutrition et parasitisme par les nématodes gastro intestinaux. Elle est ajoutée à la fin de cette partie introductive sous la partie « Etude Bibliographique Personnelle ».

Pour éviter les répétitions, et comme pour la partie 3.2.3, nous ne présenteront ici qu'un résumé des divers travaux portant sur les effets anthelminthiques des plantes à tanins, en faisant références à des tableaux et figures plus détaillées incluses dans le chapitre d'*Adv. Parasit*.

Depuis deux décennies, les effets des tanins condensés (TCs) sur le parasitisme GI des ruminants ont été abondamment étudiés car la consommation de plantes riches en

TCs semble représenter une méthode alternative ou complémentaire à l'utilisation d'AHs de synthèse pour la maîtrise des nématodes GIs (Hoste et al., 2012; Hoste et al. 2015).

L'intérêt premier sur les plantes à tanins a porté sur des légumineuses fourragères tempérées, telles les lotiers pédonculé (*Lotus pedunculatus*) et corniculé (*Lotus corniculatus*), le sulla (*Hedysarum coronarium*), le sainfoin (*Onobrychis viciifolia*), la sericea lespedeza (*Lespedeza cuneata*) et la dorycnie (*Dorycnium rectum*). Ces légumineuses ont en commun une teneur modérée en tanin condensés (TCs) (Mueller-Harvey, 2006). Elles sont dépourvues de tanins hydrolysables (THs) ce qui a permis de suspecter le rôle des TCs dans les effets observés et de prévoir une toxicité limitée.

Par la suite, des études se sont intéressées aux effets de plantes d'autres familles botaniques, en zones tempérées ou tropicales, consommées naturellement par les ruminants (Ademola et al., 2007 ; Diaz Lira et al., 2008 ; Moreno-Gonzalo et al., 2013a, 2013b). Par contre ces plantes contiennent souvent un mélange complexe de TCs, de THs et d'autres polyphénols. Les propriétés antiparasitaires ont été examinées par des méthodes *in vitro* et *in vivo*. Le rôle des tanins dans les effets observés a été souligné par l'emploi d'inhibiteur spécifiques des tanins et des flavonoïdes: le polyéthylène glycol (PEG) ou le polyvinylpyrrodilone (PVPP)

5.1. Etudes *in vitro* : mises en évidence des effets AHs

Des multiples études *in vitro* ont été réalisées afin d'évaluer l'efficacité d'extraits de plantes riches en tanins, ainsi que pour incriminer le rôle des TCs dans les effets AHs observés (Tableau 2, *Adv. Parasit.*)(Figure 7).

Les tests *in vitro* appliqués pour évaluer les propriétés des TCs sur les nématodes GIs sont ceux développés initialement pour tester l'efficacité des AHs de synthèse (Coles et al., 1992 ; Wood et al., 1995) (Tableau 1, *Adv. Parasit.*). Ces tests ont plusieurs avantages: i) ils permettent un screening standardisé de nombreux échantillons, ii) ils sont reproductibles, sensibles et fiables, iii) ils permettent de réaliser des études quantitatives (effet-dose) et qualitatives, enfin, iv) certains de ces tests permettent le calcul de valeurs inhibitrice 50 % (EC50) ce qui permet ensuite de comparer les effets d'un même extraits sur des nématodes différents ou de comparer l'activité d'extraits différents sur une même souche de nématode (Jackson et Hoste, 2010).

Chronologiquement, l'étude des effets AH des TCs sur les nématodes, a d'abord concerné l'évaluation d'extraits totaux, généralement acétoniques, de plantes riches en tanins sur différentes espèces de nématodes GIs rencontrées chez les petits ruminants *(H. contortus, T. colubriformis et T. circumcincta)* et divers stades parasitaires (œufs, L3s, adultes). Ces extraits ont été obtenus sur 3 grandes catégories de plantes : des Légumineuses tempérées, des légumineuses de zones tropicales et des arbustes et plantes ligneuses, auxquelles on peut ajouter certains sous produits des industries agro alimentaires. Les résultats des essais sont résumés dans le Tableau 2 de l'article *Adv. Parasit*.

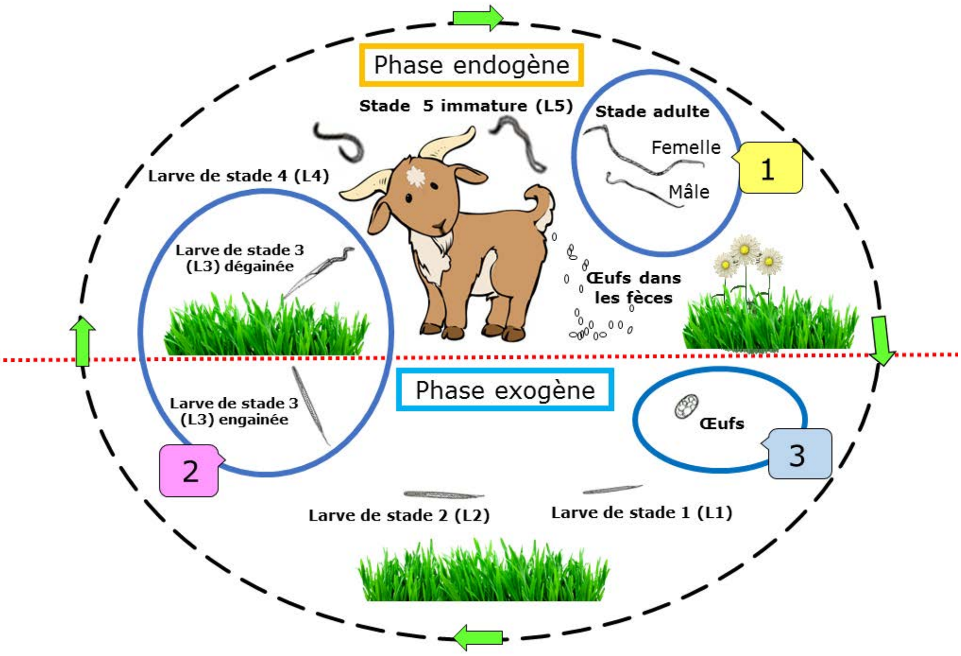


Figure 7. Principales méthodes *in vitro.*

Résume des principales méthodes *in vitro* utilisées pour évaluer l'activité AH des TCs contre les nématodes chez les ruminants. 1= les stades cibles sont les vers adultes, test AMIA (*adult motility inhibition assay*), méthode d'inhibition de la motilité des adultes. 2= le stade cible est la L3, LEIA (*larval exsheathment inhibition assay*), méthode d'inhibition du dégainement larvaire ; LMIA (*larval motility inhibition assay*), méthode d'inhibition de la motilité larvaire. 3= les stades cibles sont les œufs, et leur développement jusqu'à la larve, EHA (*egg hatch assay*), méthode d'inhibition d'éclosion des œufs ; LFIA (*larval feeding inhibition assay*), méthode d'inhibition de nutrition des L1 ; LDIA (*larval development inhibition assay*), méthode d'inhibition du développement larvaire.

Les concentrations appliquées lors des tests *in vitro* avec des extraits correspondent aux gammes de concentration en tanins mesurées *in vivo* (Molan et al., 2003 ; Terrill et al., 1994). Néanmoins, les résultats *in vitro* ne peuvent être une prédiction des effets AHs *in vivo*, étant donné qu'ils sont obtenus en dehors du contexte physiologique et immunologique rencontré chez l'hôte. Ceci justifie la nécessité de vérification par des études *in vivo*.

Par ailleurs, ces essais *in vitro* ont aussi fourni les outils permettant d'analyser le rôle des tanins et des flavonoïdes sur les vers. Ils ont ainsi conduit à mieux comprendre le mode d'action des polyphénols sur les nématodes. Il faut souligner que les résultats acquis *in vitro* reposent sur l'hypothèse d'un effet direct des TCs sur les vers (cf. infra l'article *Adv. Parasit.*).

Dans le but de préciser le rôle les tanins dans les effets observés, des inhibiteurs de tanins ont été ajoutés aux mêmes extraits, (Azuhnwi et al., 2013 ; Bahuaud et al., 2006 ; Brunet et al., 2008;). De manière plus rare, le rôle des TCs dans les effets AHs a parfois été confirmé par l'emploi de TCs purifiés (Molan et al., 2004) ou de monomères constitutifs (flavan-3-ols) des TCs (Brunet et Hoste, 2006). Les études les plus récentes ont été focalisées sur l'évaluation des fractions purifiées de tanins, afin de comprendre les interactions entre les diverses structures moléculaires des TCs et les effets AH (Naumann et al., 2014 ; Novobilský et al., 2013 ; Williams et al., 2014).

Pour préciser les effets des diverses classes de TCs, des études sur *T. colubriformis*, et *T. circumcincta* ont mis en évidence que les monomères de TCs, les flavan-3-ols et les gallates de flavanols, inhibaient significativement les valeurs d'EHA, de LDA et de LMI (Molan et al., 2003 ; Molan et al., 2004). De telles inhibitions se sont révélées dépendantes de la concentration en flavan-3-ols appliquée. Le degré d'inhibition paraître varier aussi en fonction de la structure chimique des monomères puisque les plus fortes inhibitions ont été observées pour les monomères des PDs et les gallates (Molan et al., 2003 ; Molan et al., 2004).

En bilan, ces études *in vitro* ont démontré l'effet AH des plantes riches en TCs sur les trois principales espèces de nématodes GIs (*H. contortus, T. circumcincta, T. colubriformis*) majoritairement trouvées chez les petits ruminants. Le rôle des TCs dans ces effets a également été largement corroboré. Par ailleurs, des variations d'effets ont été décrites en fonction de la plante-source de TCs et du stade parasitaire. Toutefois, il était nécessaire de confirmer en conditions *in vivo* la validité de ces résultats *in vitro*. Ces études ont d'abord été menées chez des animaux expérimentalement infestés puis vérifiées en condition d'infestations naturelles.

5.2. Etudes *in vivo* : la vérification des activités AH des TCs

5.2.1. Etudes in vivo en condition d'infestations expérimentales

La mise en place d'études en conditions d'infestations expérimentales ont permis de préciser les effets des TC sur les principales espèces parasites du tube digestif des ovins et des caprins (genre *Haemonchus, Teladorsagia* et *Trichostrongylus*) ou des bovins (genre *Ostertagia* et *Cooperia)*. Ce type d'études a aussi conduit à préciser chez l'hôte les répercussions de l'ingestion de tanins sur les divers stades clés du cycle biologique des Nématodes (éclosion et développement des œufs, larves 3 infestantes, vers adultes) par un choix approprié des périodes de distribution des nutricaments contenant des tanins.

Les principaux résultats obtenus sont mentionnés dans la légende de la Figure 8. Le détail des principaux résultats obtenus en conditions confinées avec des plantes d'origine géographique et de famille botanique diversifiées sont donnés dans le Tableau 3 de l'article *Adv. Parasit.*

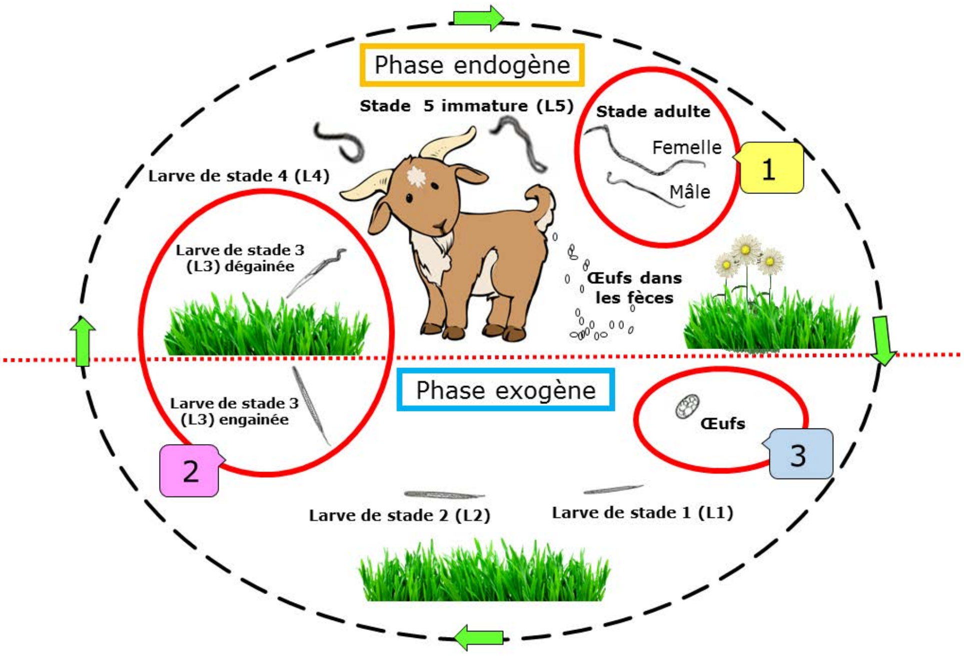


Figure 8. Etudes *in vivo*

Les études *in vivo* ont montré des effets des TCs sur différents stages parasitaires : 1) diminution des œufs excrétés pour les vers adultes, soit par diminution de nombres de vers ou baisse de fertilité des vers femelles ; 2) diminution de l'établissement des L3 dans le tube digestif de l'hôte (jusqu'à -70%) ; 3) diminution du développement des œufs jusqu'à les stages larvaires.

5.2.2. Etudes in vivo en condition d'infestations naturelles

Ces études sont moins nombreuses que les précédentes et l'interprétation de leurs résultats est plus difficile en raison: 1) de la présence de plusieurs populations de nématodes d'espèce différentes tout au long du tractus digestif, 2) de niveaux d'infestations non maitrisés et fortement variables, 3) de possibles interactions entre populations de parasites. Par ailleurs, en termes de conséquences physiopathologiques, des effets cumulatifs sont souvent soupçonnés et la prise en compte des réponses immunologiques de l'hôte est difficile à évaluer.

La question de la diversité des espèces parasitaires rencontrées peut en partie être résolue par l'emploi de méthodes helminthologiques adaptées (ex coprocultures après coproscopie et surtout bilans parasitaires après autopsie) et de paramètres physiopathologiques relativement spécifiques (*e.g.* mesure d'hématocrite marqueur de l'intensité des infestations par *H. contortus*). En l'absence de variables quantitatives vérifiées et équilibrées au début des essais et de bilans nécropsiques en fin d'essai, l'intreprétation des résultats reste délicate.

Toutefois, ce type d'étude présente un intérêt majeur qui est de se rapprocher des modes de conduite observées dans les divers systèmes d'élevage et d'explorer comment des modes diversifiés de distribution des nutricaments module, à des degrés variables, la dynamique des infestations (cf. article *Adv. Parasit.*). Les enseignements retirés sont importants pour préparer les futures utilisations en élevages.

Comme pour les études *in vivo* en conditions expérimentales d'infestation, les principaux résultats sont résumés dans le Tableau 4 de l'article *Adv. Parasit*.

5.3. Effets des tanins condensés sur la résilience des animaux

Dans de nombreuses études en conditions expérimentales, à côté d'effet sur la biologie des populations de vers, la consommation des plantes contenant des TCs a été associée à une amélioration de la résilience de l'hôte par rapport à des animaux témoins recevant une ration sans tanins (Hoste et al., 2015). Ces effets bénéfiques ont été mesurés au travers d'un meilleur statut clinique des animaux (diarrhées moins sévères, nombre de traitements AHs nécessaires, moins de mortalité) (Min et al., 2005), de mesures pathophysiologiques, notamment l'hématocrite, moins dégradés (Paolini et al., 2005a, 2005b) ou portant sur les niveaux de production comme le gain de poids (Niezen et al., 1995), la production de lait (Hoste et al., 2005a) ou de laine (Niezen et al., 1998a).

5.4. Modes d'action des tanins condensés sur les nématodes gastro-intestinaux

De manière schématique, deux hypothèses, non exclusives, sont généralement évoquées pour expliquer les effets des TCs sur les nématodes GIs des ruminants: i) un effet indirect par amélioration de la réponse immunitaire de l'hôte contre les vers; ii) un effet direct, de type pharmacologique, lié aux propriétés propres des TCs sur les nématodes. Les données fondant l'hypothèse d'un effet direct des TCs sont beaucoup plus abondantes que celles confortant un mécanisme indirect.

5.4.1. Effet indirect des tanins condensés

Chez les ruminants, en raison de leur capacité à former des complexes avec les macromélcules et notamment les protéines, la présence de quantité modérée de TCs dans la ration contribuerait à protèger les protéines alimentaires des dégradations enzymatiques du rumen et entraînerait, par conséquent, une meilleure absorption intestinale d'acides aminés (Coblentz and Grabber, 2013). Il est connu qu'une supplémentation de la ration en protéines contribue à améliorer la réponse immunitaire de l'hôte contre les nématodes GIs et à modifier la biologie des vers (Houdijk et al., 2012 ; Kidane et al., 2009).

En conséquence, l'effet des TCs sur les nématodes pourrait s'expliquer en partie, par l'amélioration de la réponse immunitaire (Hoste et al., 2012). Par ailleurs, les légumineuses fourragères ont souvent une bonne valeur nutritive, ce qui peut aussi expliquer les meilleures performances observées chez les ruminants parasités lorsque ceux-ci consomment du sulla, du sainfoin ou des lotiers (Githiori et al., 2006 ; Tzamaloukas et al., 2006).

5.4.2. Effet direct des tanins condensés

La seconde hypothèse proposée pour expliquer les effets AHs des TCs est soutenue par les nombreux résultats *in vitro*, puisque dans de telles conditions, il n'y a aucune intervention de facteurs liés à l'hôte (Hoste et al., 2012 ; Min et al., 2003). En général, ces études ont montré que les TCs affectent les nématodes GIs quelques soit l'espèce, même si des variations d'effet selon le stade parasite ou la source de TCs existent.

Par ailleurs, certains résultats d'études expérimentales *in vivo,* menés sur des durée courte et chez de jeunes animaux montrant une faible réponse immunitaire, viennent aussi corroborer cette hypothèse d'un effet de type pharmacologique des TCs sur les vers (Athanasiadou et al., 2001 ; Athanasiadou et al., 2005 ; Cenci et al., 2007 ; Kahiya et al., 2003 ; Paolini et al., 2003a, 2003b ; Shaik et al., 2006 ; Wanghorn, 2008 ; Waghorn et al., 2006).

Cet effet direct s'expliquerait par la capacité des TCs à se lier aux protéines et, par conséquent à en modifier les propriétés physiques et biochimiques. Les TCs interagiraient avec les nématodes GIs en se fixant aux macromolécules de la cuticule des vers ou de la gaine des L3s (contenant des protéines riches en proline et hydroxyproline) ou se fixeraient aux enzymes secrétées par les vers et bloqueraient ainsi leur activité (Hoste et al., 2006 ; Min et al., 2003 ; Molan et al., 2003). Il a aussi été envisagé que les interactions entre TCs et protéines de l'exosquelette (la cuticule), le tube digestif voire les structures génitales des vers affecterait certaines fonctions essentielles comme la nutrition ou la reproduction (Kahn et Diaz-Hernandez, 2000 ; Molan et al., 2003 ; Molan et al., 2004). Ces hypothèses sont confortées par des études en microscopie électronique à balayage ou à transmission sur les larves infestantes (Brunet et al., 2011) ou les vers adultes (Martínez-Ortíz-de-Montellano et al., 2010 ; 2013) obtenus avec le sainfoin ou une Légumineuse tropicale (*Lysiloma latisiliquum*).

6. Variabilité des effets antiparasitaires des tanins condensés

Globalement, les effets AHs associés à la consommation de TCs en conditions naturelles ou expérimentales ont été confirmés par de nombreuses équipes de recherche dans le monde utilisant comme modèle diverses ressources contenant des TCs (Cf Tableau 3 et 4, de l'article *Adv. Parasit*.). Toutefois, ces résultats ont aussi illustré des disparités dans les effets constatés. Celles-ci ont été associées à trois catégories de facteurs liés i) à l'hôte, ii) aux ressources botaniques exploitées et iii) aux parasites (l'espèce ou stade parasitaires).

Comprendre l'origine de cette variabilité de résultats est un des points essentiels à résoudre pour envisager une utilisation pertinente des nutricaments en élevages.

6.1. Facteurs liés à l'hôte

Les résultats de quelques études suggèrent des différences d'efficacité des plantes contenant des tanins en fonction de l'espèce hôte, en particulier entre ovins et caprins. Ainsi, dans le cas d'infestations expérimentales par *T. colubriformis*, l'administration de quebracho a été associée à une réduction de la charge parasitaire chez les ovins (Athanasiadou et al., 2000) mais pas chez les caprins (Paolini et al., 2003b). De même, en infestations naturelles, la consommation de sulla frais a induit une réduction de la charge parasitaire chez les ovins (Niezen et al., 1995, 1998a) mais pas chez les caprins (Pomroy et Adlington, 2006). Pour une même source de TCs, un effet AH existerait donc chez les ovins mais pas chez les caprins. Par contre, que ce soit en infestations naturelles ou expérimentales, l'ingestion de sainfoin (*O. viciifolia*) a causé une diminution de parasitisme chez le caprins (Paolini et al., 2005b) ainsi que chez les ovins (Manoralaki et al., 2010). Toutefois, il faut souligner qu'aucune étude n'a directement réuni caprins et ovins.

Une explication possible pour de telles différences selon l'hôte pourrait être associée à des différences physiologiques entre les ovins et les caprins, en lien avec des différences de comportements alimentaires (brouteurs vs cueilleurs) (Hoste et al., 2010). Par des adaptations physiologiques en raison de l'ingestion répétée de TCs, les ruminants cueilleurs seraient capables d'ingérer de plus grande quantité de TCs que les brouteurs en raison d' une diminution de quantité de TCs libres dans le tractus digestif, due à leur piégeage par les protéines salivaires ou à leur inactivation par les bactéries ruminales (Alonso-Díaz et al., 2010; Patra and Saxena, 2011; Salem et al., 2013; Vargas-Magaña et al., 2013).

6.2. Facteurs liés aux sources de tanins condensés exploitées

A la fois, la quantité et la qualité des TCs présents apparaissent comme des facteurs déterminants pour leur activité.

6.2.1. *Facteurs quantitatifs*

De très nombreux résultats *in vitro* ont souligné l'importance de la concentration en TCs dans les effets AHs. De manière générale, des effets dose-réponse avec les extraits ont été obtenus (exemple Athanasiadou et al., 2001 ; Molan et al., 2000 ; Paolini et al., 2004) quelque soit le type d'essai exploité. De manière similaire, un effet dose-réponse a aussi été décrit par Molan et al. (2003) concernant l'effet des flavan-3-ols, les monomères des TCs, sur le développement des œufs et la migration des L3s de *T. colubriformis*.

En 2002, une méta-analyse des résultats des premières études *in vivo* a permis d'établir une relation dose/effet entre la teneur en TCs des sources exploitées et l'effet sur l'excrétion des œufs de nématodes (Min et Hart, 2002).

Quelques études *in vivo* en conditions expérimentales ont fourni la démonstration du rôle de la teneur en TCs des rations et des quantités ingérées. Les premières données ont été obtenues par Athanasiadou et al. (2001) indiquant une relation entre la dose ingérée de quebracho et les effets chez des ovins infestés par *T. colubriformis* et *Nematodirus battus*, puisque le degré de réduction du niveau d'OPG et de la charge parasitaire était proportionnel à la dose de quebracho dans la ration. Par la suite, des études *in vivo* portant sur du foin ou des granulés déshydratés de *Lespedeza cuneata* ont confirmé une relation entre la quantité des TCs consommés par des chèvres et les effets anthelminthiques (Burke et al., 2011 ; Terrill et al., 2009). Enfin une étude fondée sur du foin de sainfoin a établi une relation dose réponse en fonction de la proportion de sainfoin dans la ration sur l'installation des Larves infestantes (Brunet et al., 2007).

6.2.2. *Facteurs qualitatifs*

La nature (qualité) des TCs au travers de leur structure (type de liaisons inter-flavaniques) et de leur composition biochimique (nature des monomères constitutifs influerait également sur les propriétés AHs.

Molan et al. (2003, 2004) ont été les premiers auteurs à suggérer que la structure chimique des flavan-3-ols, (les monomères constitutifs des TCs), serait un facteur important modulant les effets AHs. Le niveau d'inhibition serait corrélé au nombre de groupements phénoliques présents sur le cycle B des monomères (Molan et al., 2003). Les flavan-3-ols composant les prodelphinidols (PDs) (tri-hydroxylés) se sont avérés plus actifs sur les L3s que ceux des procyanidols (PCs) (di-hydroxylés) (Brunet et Hoste, 2006 ; Molan et al., 2003). En particulier, les espèces botaniques présentant des ratios PD/PCs élevés seraient les plus actives contre les vers (Hoste et al., 2006 ; Min et Hart, 2002 ; Molan et al., 2003a ; Molan et al., 2004). Par contre, le ratio des conformations *cis*/*trans* est apparu sans influence sur l'activité AH (Molan et al., 2003). Waghorn (2008) a également évoqué des relations entre la structure et la composition des TCs et leur réactivité vis-à-vis de nématodes GI.

Il est connu que le niveau de complexation des TCs avec des macromolécules, notamment les protéines, dépendrait du PM (et donc, du degré de polymérisation) et de la conformation des TCs (Lorenz et al., 2014 ; Mueller-Harvey, 2006 ; Patra et Saxena, 2011 ; Stojadinovic et al., 2013) Des phénomènes similaires seraient impliqués dans la liaison des TCs avec des protéines constitutives des larves 3 ou des nématodes adultes et expliqueraient les variations d'activité en fonction de la qualité des TCs.

Ces résultats et les hypothèses associées expliqueraient les différences d'effets observées entre deux légumineuses fourragères ayant des teneurs en TCs similaires mais différant par la nature des TCs. Ainsi, des études suggèrent que le lotier pédonculé serait plus actif contre les nématodes que le lotier corniculé (Molan et al., 2000 ; Niezen et al., 1998a). Cette différence aurait plusieurs origines. D'une part, les TCs des lotiers pédonculé et corniculé différent par leurs masses PMs (respectivement 2200 et 1900 Das) et par la nature de leur monomère terminal (respectivement un épigallocatéchol (PD) et un épicatéchol (PC). Par ailleurs, ces deux espèces de lotiers présentent des ratios PD/PCs différents (respectivement 70:30 et 27:73) (Foo et al., 1996, 1997; Min et al., 2003). De même, l'activité AH du sainfoin s'expliquerait par un ratio PD/PC élevé (de l'ordre de 70:30) et la présence d'un monomère de PDs en position terminale (Barrau et al., 2005 ; Koupai-Abyazani et al., 1993 ; Marais et al., 2000).

Enfin, à côté d'activités AH montrées pour plusieurs classes de flavonoïdes impliqués dans les voies métaboliques des TCs (cf. Tableau 3 de la section 4.2.2. et dans l'article *Adv. Parasit.*), des études récentes ont commencé à considérer de possible interactions entre tannins et flavonoïdes. Kozan et al. (2013) ont vérifié *in vitro* le rôle joué par certains flavonoïdes (luteolin-7-O-β-gluco- pyranoside et quercetin-3-O-β-glucopyranoside) et l'activité anthelminthique d'une légumineuse tempérée (*Vicia pannoica* var. *purpurascens*) sur *Trichostrongylus* spp. chez des ovins.

6.2.3. Conséquences sur l'exploitation des nutricaments en élevages

Analyser et comprendre le rôle des facteurs quantitatifs et qualitatifs des tannins qui influent sur l'activité AH est déterminant pour le choix des plantes ou variétés bioactives les plus pertinentes à exploiter ou pour le mode de distribution aux animaux à privilégier. Au travers d'un large panel d'études, plusieurs modes de distribution, reposant sur des processus technologiques variés, dans l'emploi des nutricaments ont été considérés :

1) en pâturage direct sur des légumineuses cultivées (Athanasiadou et al., 2005 ; Burke et al., 2012 ; Min et al., 2004 ; Thamsborg et al., 2003 ; Tzamaloukas et al., 2005)

2) par exploitation de parcours végétatifs diversifiés (Kabasa et al., 2000 ; Torres-Acosta et al., 2012)

3) distribué en fourrage frais (Heckendorn et al., 2007 ; Niezen et al., 1998b ; Waghorn et al., 2006), ou conservé sous forme de foin (Heckendorn et al., 2006 ; Hoste et al., 2005a ; Lange et al., 2006 ; Paolini et al., 2003b, 2005a; Shaik et al., 2006), ou d'ensilage (Heckendorn, 2007 ; Heckendorn et al., 2006)

4) sous forme de granulés déshydratés (Burke et al., 2011 ; Terrill et al., 2007)

5) administré sous forme d'extraits (Athanasiadou et al., 2000, 2001; Cenci et al., 2007 ; Dawson et al., 2011 ; Paolini et al., 2003a, 2003b).

Une conservation optimale des TCs initialement présents dans la plante fraiche en fonction des processus technologiques est recherchée pour préserver l'efficacité contre les nématodes GIs (Rochfort et al., 2008).

6.3. Facteurs liés aux nématodes gastro-intestinaux.

Par comparaison aux AH de synthèse, une des caractéristiques des nutricaments contenant des TCs (Hoste et al., 2015) est 1) de ne pas conduire à des niveaux d'efficacité de 99,9 % évitant ainsi le développement rapide de résistance aux TCs dans les populations de NGIS; 2) d'avoir des effets diversifiés sur les principaux stades parasitaires du cycle biologique (cf. Figure 7 de la section 5.2.1.); 3) d'avoir des effets diversifiés en fonction des genres/espèces de nématodes parasites impliqués.

6.3.1. En fonction du stade parasitaire.

Des variations d'efficacité ont été décrites en fonction du stade parasitaire, au travers d'études *in vitro* ou *in vivo* comparant les effets des TCs sur les œufs, les L3s et les vers adultes de la même espèce de vers. Par exemple, dans une étude *in vitro* sur le sainfoin, les L3s sont apparues plus sensibles à l'effet des TCs que les vers adultes puisque les extraits inhibaient la migration larvaire mais pas la mobilité des vers adultes de *T. colubriformis* (Paolini et al., 2004). Par ailleurs, les L3 de *T. colubriformis* semblent plus sensibles que les œufs aux extraits de bruyère (*Erica* spp) (Moreno-Gonzalo et al., 2013a).

Ces variations d'effet en fonction du stade parasitaire ont également été retrouvées dans des études *in vivo*. Chez les caprins, l'administration de quebracho n'a eu aucune incidence létale sur des populations de vers adultes alors qu'elle a été associé à une forte réduction d'installation des L3s lorsque l'infestation a coïncidé avec l'ingestion des TCs (Paolini et al., 2003b). Un résultat similaire a été trouvé chez des ovins consommant du sulla (Tzamaloukas et al., 2005).

Ces différences de sensibilité pourraient s'expliquer par des diversités de protéines ou de glycoprotéines constitutives entre les œufs, les L3 et les vers adultes et en conséquence, des modes d'action divergents des TCs.

6.3.2. En fonction de l'espèce parasitaire

Un des avantages des familles d'AH de synthèse est d'être « à large spectre » c'est-à-dire de montrer une activité multivalente sur les différentes espèces de nématodes parasites des divers organes du tube digestif.

Le concept de nutricaments et les objectifs associés diffèrent de celui des AHs de synthèse (Hoste et al., 2015). Toutefois, une bioactivité des PSMs sur un large spectre de nématodes correspondant à divers organes anatomiques est à rechercher.

Les résultats d'études *in vivo* ont montré des divergences d'effets selon les espèces en cause, en particulier entre espèces parasites de l'abomasum ou de l'intestin grêle (Hoste et al., 2006, 2012). Deux hypothèses ont été proposées pour expliquer ces différences : 1) soit des différences spécifiques intrinsèques; 2) soit, en supposant un mode d'action direct des TCs et des flavonoïdes associés sur les nématodes, des différences d'exposition aux polyphénols bioactifs dans chaque organe digestif en fonction des conditions locales d'environnement. Pour exemple, la disponibilité des TCs sous forme libre dépend du pH. La stabilité des complexes 'TCs-protéines' est favorisée à des pHs neutres proches de 7 (dans le rumen puis l'intestin) et sont défavorisés aux pH acides (dans l'abomasum) (Bruneton, 1999 ; Perez-Maldonado et Norton, 1996 ; Terrill et al., 1994). Le pH acide de l’abomasum induirait ainsi une libération des TCs, fixés aux protéines salivaires ou alimentaires (Butter et al., 1999), les rendant disponibles pour perturber la biologie des vers.

En raison de l’importance des questions liés à l’analyse des facteurs dépendant des Nématodes parasites, une étude bibliographique a été menée pour examiner les deux hypothèses sur la base des données publiées, en prenant comme modèle de comparaison l’espèce *Haemonchus contortu*s en raison de ces spécificités au regard de sa biologie, de son pouvoir pathogène et des difficultés de maitrise particulières liés à la prévalence élevée de résistance aux AHs de synthèse pour cette espèce. Cette étude fait suite à cette introduction.

LES OBJECTIFS

A l'échelle mondiale, les études concernant les effets de plantes riches en métabolites secondaires (tanins condensés) sur le parasitisme gastro-intestinal par des nématodes chez les petits ruminants se multiplient. Les résultats indiquent souvent des effets favorables de ces nutricaments, en raison de conséquences négatives sur les populations de vers ou de répercussions favorables sur la réponse de l'hôte aux infestations.

Néanmoins, une forte variabilité dans les résultats a été observée en fonction des ressources exploitées (variations des quantités ou de la qualité des tannins présents), de l'hôte et des espèces parasitaires impliquées (notamment celles de l'abomasum vs. des intestins).

L'objectif général de notre travail de thèse vise à une meilleure compréhension des relations existant entre la structure (qualité) des tanins condensés et leur activité contre les nématodes parasites des petits ruminants. Nos travaux ont été réalisés en prenant comme modèle, *Haemonchus contortus*, un nématode de l'abomasum. La prise en compte des spécificités d'effets des TCs selon l'espèce parasite a été évaluée en comparant les résultats obtenus sur *H. contortus* avec ceux acquis sur *Trichostrongylus colubriformis*, une espèce intestinale. Le sainfoin (*Onobrychis viciifolia*) a été notre modèle de plante riche en TCs.

Le premier objectif de notre travail s'est focalisé sur l'étude des relations entre structure des TCs et activité antihelminthique. Pour ce faire, les effets antiparasitaires d'une gamme de fractions purifiées de TCs d'origine botanique diversifiée, choisies en raison des différences de concentration et de structure des tannins ont été mesurée à l'aide d'un test *in vitro* de référence. Ces essais ont été réalisés à la fois sur des larves d'*H. contortus* et de *T. colubriformis* pour prendre en compte d'éventuelles spécificités parasitaires dans les réponses (Article 1). Dans une deuxième étape, sur le même modèle expérimental (*H. contortus)*, nous avons aussi évalué l'existence de possibles interactions entre TCs et flavonoïdes chimiquement proches sur les propriétés anthelminthiques (Article 2).

Le deuxième objectif de ce travail a été de confirmer *in vivo* l'existence de relation structure/activité des TCs et bioactivité sur les nématodes. Dans ce but, l'efficacité de deux sources de TC, caractérisées par des structures chimiques divergentes a été évaluée sur la biologie des vers et sur la résilience d'ovins expérimentalement infestés par *H. contortus* et *T. colubriformis* (Article 3). Au cours de ce même protocole expérimental, nous avons mesuré les variations de quantité et de qualité (structure) des TC, issus des deux ressources, le long du tube digestif des ovins. Ceci afin de répondre au **troisième objectif** de notre travail qui était de déterminer si les variations d'effets constatés selon les espèces de Nématodes pouvait s'expliquer par des variations d'exposition aux tannins biodisponibles en fonction de l'organe parasité (Article 4).

Cette thèse a été préparée sous forme d'articles soit publiés soit prêts pour soumission.

ETUDE BIBLIOGRAPHIQUE PERSONNELLE

CHAPITRE 1 : Meta analyse des données qui évaluent les interactions entre la nutrition et les infestations par *Haemonchus contortus* (et autres nématodes gastro-intestinaux) des petits ruminants

ARTICLE : Interactions entre la Nutrition et les Infestations par *Haemonchus contortus* (et d'autres nématodes gastro-intestinaux) des petits ruminants

HOSTE, H., TORRES-ACOSTA, **J. QUIJADA**, I. CHAN PEREZ, M. M. DAKHEEL, D.S KOMMURU, I. MUELLER HARVEY, T.H. TERRILL.

2016. *Adv. Parasit.* (accepté)

Interactions between Nutrition and Infections with *Haemonchus contortus* and Related Gastrointestinal Nematodes in Small Ruminants

Hervé Hoste [1,2*], Juan F.J. Torres-Acosta [3], Jessica Quijada [1, 2], Israel Chan-Perez[3], Mohammed M. Dakheel [4], Dill S. Kommuru [5], Irène Mueller Harvey [4], Thomas H. Terrill [5].

[1] INRA, UMR 1225 IHAP, 23 Chemin des Capelles, Toulouse F-31076, France
[2] Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France
[3] Campus de Ciencias Biológicas y Agropecuarias, FMVZ, Universidad Autónoma de Yucatán, Km 15.5 Carretera Mérida-Xmatkuil, Merida, Yucatan, Mexico.
[4] University of Reading, School of Agriculture, Policy and Development, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom
[5] Fort Valley State University, 1005 State University Drive Fort Valley, GA 31030, USA

Contents

---

* Corresponding Author: (H.H.) E-mail address: h.hoste@envt.fr

Abstract

Interactions between host nutrition and feeding behaviour are central to understanding the pathophysiological consequences of infections of the digestive tract with parasitic nematodes. The manipulation of host nutrition provides useful options to control gastrointestinal nematodes as a component of an integrated strategy. Focused mainly on the *Hameonchus contortus* infection model in small ruminants, this chapter (i) illustrates the relationship between quantitative (macro- and micro-nutrients) and qualitative (plant secondary metabolites) aspects of host nutrition and nematode infection, and (ii) shows how basic studies aimed at addressing some generic questions can help provide solutions, despite the considerable diversity of epidemiological situations and breeding systems.

***Keywords**:* Pathophysiology, Nematode, Nutraceutical, Plant Secondary Metabolites, Tannins, Resistance, Resilience, Anthelmintic*.*

Abbreviations:

AH *(anthelmintic);* ***GIN*** *(Gastrointestinal Nematodes);* ***L3*** *(infective third-stage larvae);* ***PCV*** *(packed cell volume);* ***FEC*** *(faecal egg count);* ***EPG*** *(eggs per gram);* ***PPRI*** *(periparturient relaxation of immunity)*

VFI *(voluntary feed intake);* ***MP*** *(metabolisable protein);* ***ME*** *(Metabolisable Energy);* ***NPN*** *(non-protein nitrogen);* ***UMB*** *(urea molasses blocks);* ***COWP*** *(copper oxide wire particles).*

TEM *(transmission electron microscopy);* ***SEM*** *(scanning electron microscopy);* ***SAR*** *(structure-activity relationship);* ***PEG*** *(polyethylene glycol);* ***PVPP*** *(polyvinyl poly pyrrolidone)* ***PSM*** *(plant secondary metabolites);* ***CT*** *(condensed tannins);* ***PD*** *(prodelphidins;* ***PC*** *(procyanidins);* ***SL*** *(sericea lespedeza);* ***BG*** *(Bermuda grass).*

1. INTRODUCTION

In any grazing system, from steppes or temperate grasslands to tropical forests, wild and domestic ruminants co-exist with both the forages and browses, which are a source of both nutrients and plant secondary metabolites (PSM; see Box 1), as well as with the infective larvae of parasitic gastrointestinal nematodes (GINs) associated with grazing (See Figure 1). Therefore, since ruminants consume forages and browses representing different plant communities, GINs should also be considered as normal inhabitants of grazing ruminants. In addition, ruminant hosts are able to live, reproduce and be productive with a moderate numbers of GINs in their digestive tract.

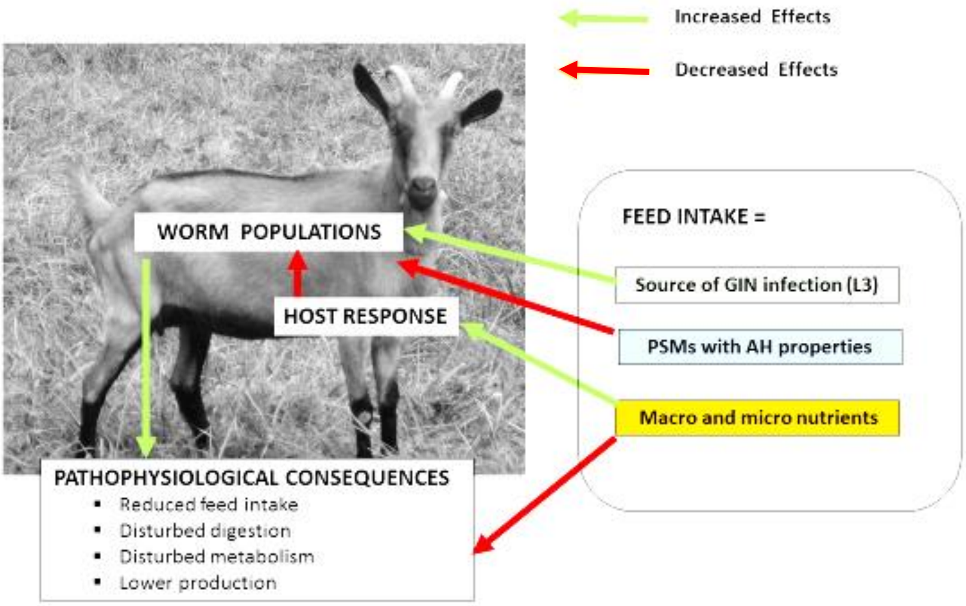


Figure 1: Relations between host nutrition and pathological and pathophysiological changes caused by GIN infections, including *H. contortus*.

The long-standing, close association between ruminants, plants and GINs has shaped several features of these different organisms. In other words, some features of plants, ruminants and their GIN parasites have co-evolved. For example, parasites are able to endure the conditions of the gastrointestinal tract of ruminants, enabling GINs to reproduce successfully and to generate offspring that will eventually invade other generations of ruminants and allow the survival of the parasite species. Ruminants, in turn, have evolved defences, such as physical barriers and immunological mechanisms (resistance) and physiological responses (resilience) (see Box 1), which enable them to regulate GINs and maintain them at sustainable levels, conserving both the hosts and their parasitic populations. Under natural conditions, it is evident that GINs are part of a negative feedback system, which is a natural process that helps in regulating the population of ruminants to limit the use of forages in a paddock. This ecological perspective of the interactions between GINs and ruminants is crucial to understanding nutrition-parasite interactions in the case of domestic ruminants.

Worldwide, most small ruminant production systems are extensive and outdoors. They rely on the use of pastures and/or browses, and, hence, are derived from the natural conditions encountered by wild ruminants. The usage of plant resources seeks to ensure the nutrition of sheep and goats in a wide variety of breeding systems. However, these resources also represent potential sources of GIN infections because of the presence of infective third-stage larvae (L3). As a result, the infections of small ruminants with GINs, including *H. contortus*, represent one of the main constraints for small ruminant production, health and welfare. Infections with GINs is a common outcome of grazing and/or browsing systems in a wide range of ecological settings, irrespective of the variability of local plant resources.

The complexity of the interactions between GIN infections, plant resources and host nutrition for sheep and goats relates directly to this ecological context, and is reflected in 3 paradoxes.

Paradox 1: The grazed and browsed plants from fields exploited by ruminants are both feed resources, in terms of nutritional value, which can help to meet the main requirements of the host. However, these plants can also be the source of L3 of GINs. Once the infective larvae are established in the digestive tract, a GIN infection disturbs the digestive physiology of ruminants and, depending on the severity of the infection, nematodes may severely impair the utilisation of nutritional resources harvested from the field.

Paradox 2: The feed exploited by domestic ruminants can provide the nutrients, which may represent a possible solution to alleviate the negative effects of GIN populations on the physiology of the host. This is the result of the macro- and/or micro-nutrients obtained from the diet, which may meet the extra nutritional requirements caused by parasitism (see Section 2; cf. Coop and Kyriazakis, 1999, 2001).

Paradox 3: The feed exploited by domestic ruminants can also be the source of bioactive plant secondary metabolites (PSMs), which can have direct anthelmintic (AH) properties. Thus, the constant consumption of feed resources containing bioactive PSMs, above a sufficient threshold of concentration and for a sufficient time, may affect/regulate the biology of different developmental stages in the life cycle of GINs (see Section 3; cf. Hoste et al., 2012, 2015)

Studies of feeding behaviour and the related voluntary feed intake (VFI) of ruminants are on-going and reflect the complexity of interactions between nutrition and GIN infections in ruminants. It has been established that the feeding behaviour of grazing ruminants might govern which plant species are eaten, how much of each plant and which plant component is consumed (González-Pech et al., 2014). The latter implies that such feeding behaviour dictates both the source of GIN infections, and also the potential route for adaptation to the quality and quantity of PSMs in a particular feedstuff. However, the feeding behaviour of ruminants can also be modified by high intensity GIN infections and by high PSM content, which complicates the scenario. A large GIN infection may cause a reduction of VFI in ruminants, which is one of the main pathophysiological effects of GIN infections (Coop and Holmes, 1996; Fox, 1993; Simpson, 2000). Moreover, a high PSM content may have negative effects on the VFI in animals. From the nutritionist's viewpoint, PSMs that reduce VFI are considered anti-nutritional compounds. The reduction in VFI caused by GIN infections and the anti-nutritional PSMs may be considered, in essence, an important part of the natural mechanisms aiming to reduce the impact of ruminants on plants. The ultimate goal of nature could be to deter herbivory, hence reducing the impact of ruminants on plants.

Evidence is now accumulating to indicate that, when infected with GINs, ruminants can modify either the amount or the type of the feed they eat; this may ameliorate the negative impact of parasitic infections, and has been described as self-medication (Hutchings et al., 2003; Villalba and Provenza, 2007; Villalba et al., 2014). Hence, the study of interactions between nutrition and GIN infections in domestic ruminants offers a model to explore the complexity of interactions between some GIN parasites and their hosts in the context of "sustainable interactions" (Combes, 1995) as well as different feedback mechanisms at the plant-animal-parasite interface. These different concepts are illustrated in Fig. 1 and provide the basis for the present chapter.

Haemonchus contortus represents a useful model to study nutrition-parasite interactions because of its worldwide distribution, its specificities in regards to pathogenic mechanisms, its haematophagous mode of feeding, and the severe pathological consequences when animals are infected with large burdens of the parasite. In addition, *H. contortus* represents one of the three main nematode genera (besides *Teladorsagia* and *Trichostrongylus* spp.) most commonly used for studies of the interactions between GIN infections and host nutrition. Besides the peculiarities of its biology (including feeding mode and high reproduction rate), its epidemiological features and its pathophysiological effects on the host, *H. contortus* populations world-wide have developed resistance to commercial anthelmintic drugs (Jackson et al., 2012; **Chapter X – in this Special Issue**). Thus, alternative control measures are being sought to develop sustainable and integrated GIN management systems (Torres-Acosta and Hoste, 2008). These aspects explain why this special issue of Advances in Parasitology is dedicated to this nematode species, many of which are covered in other chapters of this book.

Studies of the interactions between host nutrition and worm populations are warranted because of the importance of the pathophysiological effects caused by this particular worm. Therefore, the *Haemonchus* infection model in small ruminants offers numerous scientific opportunities, as it exarcerbates the consequences of infection. These opportunities stem from:

(1) The wide prevalence and geographical distribution of *H contortus* species. These chatacteristics have enabled a large number of studies focussed on the nutrition-parasite interactions using this GIN as a model in sheep and goats. Additionally, a large amount of information is available on other aspects of host-nematode interactions (**see other Chapters in this Special Issue**).

(2) A sound understanding of the pathogenic mechanisms associated with the presence of a unique anatomical feature that allows *H. contortus* to exploit the blood supply and to interfere with the blood coagulation mechanisms in the host, in order to secure its own nutrition (Rhoads and Fetterer, 1996a, b).

(3) The anaemic syndrome that results from the presence of *H. contortus* is distinct from the digestive syndromes (disturbances of digestive and absorptive processes), such as diarrhoea, which occurs in the presence of most other abomasal GIN species (i.e. *Ostertagia/Teladorsagia* or *T. axei*) and different genera of GIN (e.g., *Trichostrongylus*, *Cooperia* and *Nematodirus*) in the small intestine. These biological aspects are related to a better knowledge of the pathogenic mechanisms and pathophysiological consequences of *H. contortus* infection compared with other GINs.

(4) Highly significant correlations have been repeatedly established between adult *Haemonchus* numbers and/or worm mass (weight) as well as anaemia, which is the main pathogenic effect observed in the host animal. Anaemia can be measured by packed cell volume (PCV) and haemoglobin levels (Le Jambre, 1995), and can also be estimated by clinical signs (e.g, colour of the mucosae). This latter approach led to the development of some qualitative (although non-specific) tools for the inference of *Haemonchus* infection in sheep and goats (the FAMACHA method; Van Wyk and Bath, 2002). Laboratory and qualitative methods provide more reliable data on the dynamics of *H. contortus* infections and the effect of nutritional improvement on host resilience (see Box 1) against such abomasal infections compared with other GINs. However, the use of this type of tool to identify the anaemic syndrome can be misleading on farms with low nutritional planes. A recent study showed the difficulty in distinguishing between anaemia originating from malnutrition and that caused by *H. contortus* and/or other GIN infections (Torres-Acosta et al., 2006). The latter aspect illustrates the complexity of clinically differentiating haemonchosis from malnutrition, both of which are common in many production systems.

(5) Some of the most highly prevalent parasitic nematodes of the human digestive tract (namely *Ancylostoma* spp. and *Necator americanus*) are also blood-feeders that share several biological traits with *Haemonchus* (cf. Hotez et al., 2006; Williamson et al., 2003). Therefore, any data acquired on the interactions between host nutrition and the infection with a haematophagous nematode of veterinary importance might also aid in developing methods of control against some medically important GINs of humans.

Information on the interaction between host nutrition and GIN infections has been reviewed by various authors in the past (including Coop and Holmes, 1996; Coop and Kyriazakis, 1999, 2001; Fox, 1993; Holmes, 1993; Hoste et al., 2005; Knox et al., 2006; Knox and Steel, 1996; MacRae, 1993; Parkins and Holmes, 1989; Poppi et al., 1990; Torres-Acosta et al., 2012; Van Houtert and Sykes, 1996;). This chapter focuses on data and findings for *H. contortus* and related congeneric species (including *H. placei, H. similis* and *H. longistipes*), to illustrate two primary aspects, namely (**i**) the quantitative manipulation of nutrition to improve the host response and, **(ii)** the effect of PSMs on worm populations (see Fig. 1). A third aspect on the interactions between nutrition (and feeding behaviour) and GIN infections is only evoked because reviews have been published recently on the concept of self-medication (Villalba et al., 2014; Villalba and Provenza, 2007). Most data will refer to *H. contortus* of sheep and goats, because the vast majority of published studies refer to these two small ruminant species. Reference to *Haemonchus placei* of cattle is made when applicable. As far as possible, this chapter attempts to compare results obtained for *Haemonchus* spp. with those for other GIN genera, and will relate to (i) their main anatomical location in the abomasum (= *Haemonchus* spp. and *Teladorsagia/Ostertagia s*pp.) or in the small intestine (*Trichostrongylus colubriformis*, *T. vitrinus* and *Nematodirus* spp.), and (ii) the mode of nutrition: haematophagous for *Haemonchus* spp. *versus* non-haematophagous genera for *Teladorsagia/Ostertagia/ Trichostrongylus*/*Cooperia* and *Nematodirus* spp.

2. QUANTITATIVE ASPECTS

The rationale that underpins the quantitative manipulation of host nutrition is directly based on knowledge of the various main pathophysiological processes that result from the presence of parasitic nematodes in different digestive organs (see Figure 2). Such knowledge provides the potential for the development of complementary, alternative for improvement of GINs' control.

2.1. Pathophysiological and nutritional consequences of *H. contortus* infections

For *Haemonchus* spp. (like other GIN species in small ruminants), parasitism of the digestive tract can be described as a nutritional disease, because of the increased nutritional demands, in much the same way as lactation or pregnancy might be viewed (Poppi et al., 1990). Overall, the presence of GINs has been linked to three main effects on host digestive and general physiology. These pathophysiological consequences, illustrated in Fig. 2, explain the production losses, as well as the subclinical and clinical signs, which are usually associated with GIN infection (Coop and Holmes, 1996; Holmes, 1993; Hoste et al., 1997), providing a descriptive framework for interpreting the pathological and pathophysiological changes described in different studies of *H. contortus.* The four main pathophysiological impacts are:

(1) Reduction of feed intake (Voluntary Feed Intake = VFI). When compared with infection with the other main parasitic genera (*Teladorsagia* and/or *Trichostrongylus* species), VFI reduction in sheep or goats with subclinical *H. contortus* infection appears as milder and more transient (Bambou et al., 2009, 2013; Dakkak, 1984; Wilson et al., 1969).

(2) Decreased food digestibility due to the reduction of enzymatic functions and changes in HCl secretion that affect digestive processes in the abomasum (step 1).

(3) Disruption of the digestive processes decreases the absorptive processes in the small intestine (step 2).

(4) Nutrients absorbed via the digestive tract can be diverted from tissues, including bones, muscles, udder and/or wool follicles) to maintain blood and tissue homeostasis, in order to compensate and replace losses caused by GINs and to ensure host survival.

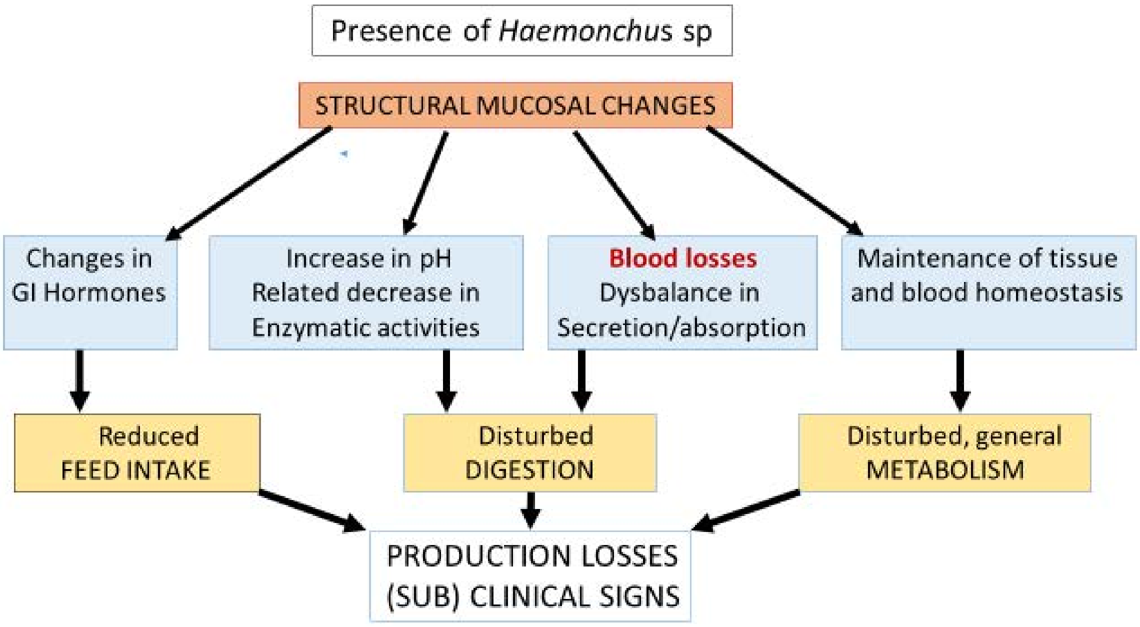


Figure 2: A summary of the main pathological and pathophysiological consequences of *H. contortus* infection in sheep and goats.

Studies of *H. contortus* infection demonstrated the specificity of the pathological changes in the host in relation to some biological traits of *Haemonchus* species, relating primarily to their blood feeding activity, and also to their high reproductive index (number of eggs produced per female worm per day) when compared with other GIN genera affecting small ruminants.

Early studies examined the key sequential pathological features caused by *Haemonchus* in ruminants, mainly sheep (Hunter and MacKenzie, 1982; Salman and Duncan, 1984, 1985) and, to a lesser extent, in goats (Al Quaizy et al., 1986; Al Zubaidy et al., 1987). The major lesions in the abomasum have been described (Simpson, 2000), and include:

- A hypertrophy of the abomasal mucosa associated with an infiltration of inflammatory cells (mast cells, eosinophils, globule leucocytes and lymphoid aggregation), particularly in the case of reinfected or immunized sheep (Salman et al., 1985);
- A general hyperplasia of mucus-producing cells, combined with an erosion of the surface epithelial mucous cells;
- A decrease in the number of functional parietal cells (HCl-producing) and replacement by non-differentiated, abnormal cells;
- For the chief cells, which produce the pro-enzyme pepsinogen, some studies indicate the maintenance of cell numbers, but they are less differentiated and functional.

The pathophysiological and pathological changes generated in the abomasal mucosae by the presence of *Haemonchus* spp. (Scott et al., 1999; Simpson, 2000) can be summarized as follows:

- Structural changes relating predominantly to the HCl-producing parietal cells can impact on pH values. Described has been an increased in abomasal pH from 2.6 to 3.6, with transient peaks of up to 6.0, depending on the course of infection (Nichols et al., 1987; Simpson et al., 2000).
- These pH changes have direct effects on the abomasal digestion step, which liberates nutrients for further digestion along the gut (small intestine). The pH increase reduces the conversion of pepsinogen (produced by the gastric chief cells) to active pepsin.
- In addition, increased abomasal pH will also favour possible invasion (colonisation) of the digestive tract with bacteria that are normally inactivated by low abomasal pH in non-infected hosts.
- Detrimental effects on crude protein digestion lead to higher protein losses, which have been recorded in *H. contortus*-infected animals.

One of the primary traits of *H. contortus* infection is anaemia due to blood feeding activity. Several studies have evaluated blood losses as a consequence of *H. contortus* infection in the host animal (Clark et al., 1962; Dargie and Allonby, 1975; Le Jambre, 1995). In a subclinical infection, red blood cell losses range from ~ 10 to 20 mL per day, which equates to ~ 50 to 100 mL of blood (Dargie and Allonby, 1975). These values correspond to an estimated 15 µL to 50 µL of blood per worm per day (Le Jambre 1995). These detrimental losses are explained by the fact that adult *Haemonchus* can suck blood for several minutes at any one site from the abomasal mucosa, and can then move to a different position to feed (Le Jambre, 1995; Nicholls, 1988), allowing blood to leak into the abomasum due to the presence of anticoagulant compounds/enzymes excreted/secreted from *H. contortus* (see Rhoads and Fetterer, 1996a and b). Blood losses are caused by fourth-stage larvae and adult worms.

Highly significant correlations have been found between worm numbers and blood loss (Le Jambre, 1995), making the packed cell volume (PCV) a valuable indirect way of estimating the number of adult *H. contortus* present in a host animal. Moreover, significant correlations were also found between blood loss and either the parasite mass or the total number of parasite eggs produced, indicating that the blood serves to ensure worm growth and reproduction (Le Jambre, 1995). In addition, by using isotopic markers and related methodologies (Abbott et al., 1985, 1986; Dargie and Allonby, 1975; Gennari et al., 1991), three phases in the pathophysiological process can be identified, depending on iron reserves available in the host animal: Phase 1 corresponds to the establishment of infection and is characterized by a regular decrease in PCV. Phase 2 corresponds to a plateau in PCV values, because of a balance between continuous blood losses in the abomasum and a stimulated erythropoiesis (compensation to ensure blood homeostasis). Finally, Phase 3 is characterized by a marked reduction in PCV and serum iron concentration (uncompensated anaemia). These phases suggest that iron depletion is the first limiting nutritional factor, explaining the severity of effects linked to *H. contortus* infection. Increased protein losses in the abomasum also occur. However, several studies have suggested the possibility of reabsorption of proteins in the small intestine in the presence of a sole or dominant *H. contortus* infection as a compensatory process (Hoste, 2001; Rowe et al., 1982).

2.2. A conceptual framework to understand and manipulate host nutrition as an aid to controlling *Haemonchus contortus* infection.

2.2.1. Importance of nutrition

"It is usually very generally held that nutritional factors exercise a vital influence on the degree of infestation of sheep with Haemonchus contortus. Here again, however, resistance to infestation is commonly confused with resistance to the effects of infestation". This statement by Clunies-Ross (1932) underscored that host nutrition has been identified as a key factor in controlling *H. contortus* infection very early in the history of modern parasitology and indicated the potential impacts on host resistance and resilience. On the one hand, dietary supplementation (increased nutritional manipulation) can provide the nutrients that contribute to tissue maintenance and/or blood homeostasis and host production, despite the presence of worms (= resilience; see Box 1). On the other hand, it can provide the nutrients needed to improve the host response by meeting the increasing demands of raising an immune response against the worms (= resistance; see Box 1), in order to regulate the worm populations in immune-competent animals. The first two outcomes (blood and tissue homeostatis) are covered as first priorities because they are essential for the survival of the host. Then, if there are more nutrients available, these extra nutrients can be used to achieve (increase) production purposes, as suggested by

Coop and Kyriazakis (1999, 2001). The latter aspect also forms part of the improvement of resilience.

The beneficial effects of nutritional manipulation of GIN control depend largely on the overall nutritional level of the animals (Figure 3). One of the most obvious statements concerning the interactions between host nutrition and GIN infections, particularly in the case of *Haemonchus* infection, is that any combination of malnutrition and digestive parasitism will lead to uncompensated pathophysiological disturbances, and, subsequently, to severe clinical signs (including a high mortality rate). Under tropical conditions, when *Haemonchus* is the dominant genus, this aspect is particularly evident on farms with alternating wet (abundance of nutrition / high parasitic risks) and dry (low nutritional value of the grazed resources / lower parasitic risk of GIN infections) seasons. As stated by Amarante (2014), *"Farmers blame the worms or the lack of highly efficient chemical anthelmintic drugs" (because of anthelmintic resistance). However the primary source of the problem is malnutrition!"* In this situation, the scarcity of resources, as defined by Coop and Kyriazakis (1999, 2001), is linked to a general lack of both macro-nutrients (protein/energy) and micro-nutrients. Under such circumstances, any improvement in nutrition will be of benefit to the host in counteracting *Haemonchus* (and other GIN) infections and related effects.

2.2.2. Targeted dietary supplementation for different nutritional components

Coop and Kyriazakis (1999, 2001) introduced **3 main concepts** to describe the overall framework of interactions between host nutrition and GIN infections (see Fig. 3). These authors proposed the following hypotheses:

a) Usually, there is one main dietary component (described as "limiting dietary factor" or "scarce resource") whose metabolism is seriously affected by the presence of GINs. Therefore, targeted corrections of this limiting nutritional factor should have major beneficial effects on the host response (both resilience and resistance) to GIN infections. Nitrogen and, to a lesser extent, energy are usually these limiting dietary resources.

b) This framework suggests that the allocation of the overall (or specific) nutritional diet resources is needed to meet the pathophysiological requirements of the parasitized host, and will depend on different priorities relating to the host physiological status. The priorities are also related to the general ecological context of the interactions between host and GIN infections (as explained in Sub-section 1.1). These priorities for the host populations are: (1) Survival (maintenance of body protein = blood and tissue homeostasis); (2) Transmission of genes to offsping: reproductive efforts (including lactation) and preservation of offspring survival = acquisition of immunity against GINs in young animals; (3) The expression of host immunity, promoting growth and reproduction in the host; (4) Storage of reserves in the host (by preservation of fat).

c) The higher the priority given to a physiological function, the less likely is that it would be affected by host nutrition. Therefore, the framework suggests that improved nutrition will initially lead to an increase in host resilience (Coop and Kyriazakis, 1999), and then to an improved host resistance.

d) Whatever the GIN species, this theoretical framework is supported by evidence from a large number of studies of meat- and wool-producing lambs and sheep (reviewed by Knox, 2003; Knox et al., 2006; Van Houtert and Sykes, 1996). There is considerably less data and information for kids and goats (Hoste et al., 2005), in particular dairy goats, as well as for cattle.

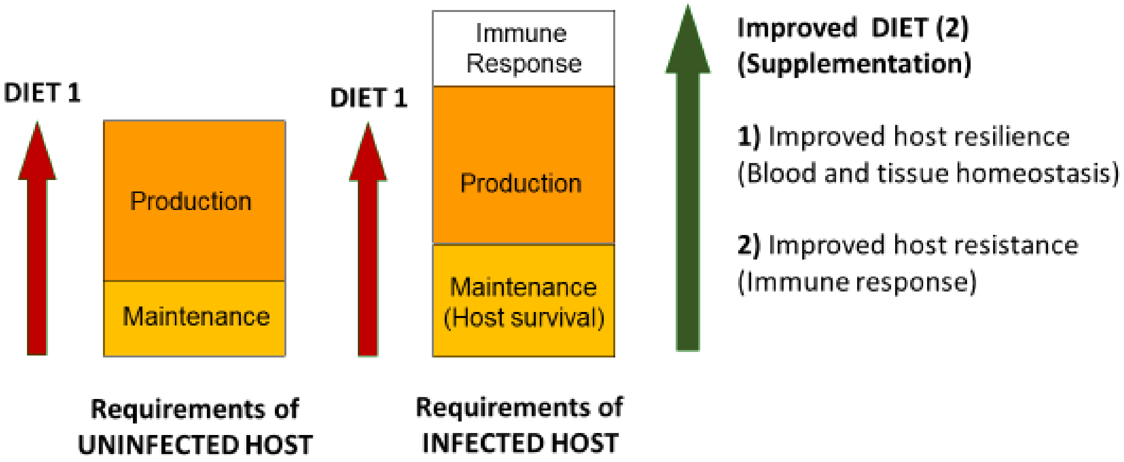


Figure 3: A schematic representation of a theoretical framework to illustrate: **1)** that a diet (red arrow) aiming at covering the nutritional requirements of a non infected host is insufficient to cover the increased requirements associated with GIN infections (e.g., increased cell turnover to maintain blood and tissue homeostasis and development of an immune response); **2)** the rationale for an increased general and/or specific diet supplementation is illustrated with the green arrow (Derived from Coop and Kyriazakis, 1999, 2001).

2.3. Supplementation with nitrogen resources to improve host resistance and resilience against *H. contortus.*

Most results from pathophysiological studies have emphasized the fact that protein metabolism is much more affected by GINs than other dietary components, such as energy (Bown et al., 1991). Most studies of nutrition-parasite interactions were undertaken in the UK, Australia and New Zealand, mainly with European breeds of sheep used for meat and wool production. Very few initial studies were performed on goats, cattle or dairy ruminants. Logically, under farming conditions in developed countries, i.e. with animals receiving a high nutritional level when grazing on high quality paddocks, the nitrogenous component is usually the main limiting factor in the ruminant diet. Therefore, early studies focused mainly on the effects of protein supply on the outcome of infection.

Most investigations of protein supplementation have been based on:

- **Pen studies (controlled infections)**: Such studies are conducted under controlled experimental infection conditions. Different infection approaches were considered, including single challenge or trickle infections with monospecific (*H. contortus*) or mixed GIN infections (*H. contortus* + another, usually intestinal nematode). Pen studies allowed analyses and detailed descriptions of the interactions between host nutrition and GIN infections. In addition, they have provided hypotheses and arguments to support the framework (Fig. 3; Coop and Kyriazakis, 1999).
- **Field studies (natural infections):** Animals are exposed to natural infections, while browsing or grazing in the field. The interpretation of results of field studies are challenging to relate directly to *H. contortus* infection. However, under tropical conditions, *H. contortus* is usually one of the predominant GINs, and often occurs with *T. colubriformis* and/or *Oesophagostomum columbianum.* The relative prevalence of each species depends on season (wet *versus* dry) and management conditions (e.g., grazing system and anthelmintic treatments used).

Although dietary protein supplementation has been shown to increase resilience and resistance to parasitism in sheep under temperate conditions, van Houtert and Sykes (1996) stressed that many studies did not rigorously assess the role of protein status of the host. These authors reported that an increased supply of dietary crude proteins was not necessarily translated into a better supply of metabolizable protein (MP) for the animal. The latter could be caused by differences in the rate of degradation of dietary crude protein in the rumen and in the conversion efficacy of dietary crude into metabolizable proteins. Furthermore, MP supply is not a fixed characteristic of diet, as it varies with feed intake (AFRC, 1993), the ruminal fermentations, the feed passage from rumen to the small intestine, and the rate of rumen escape proteins. Therefore, recent studies have emphasized the need to consider both the protein and energy components, in particular under tropical conditions (see Sub-section 2.4).

2.3.1. Effects of supplementation with different sources of dietary nitrogen in controlled pen studies

The main GIN used in controlled feeding pen studies has been *H. contortus*. This model was used to investigate the potential for improving the two components of the host response (resilience and resistance) through supplementation with different sources of rumen-escape proteins (Abbott et al., 1985a, 1986a, 1988; Israf et al., 1998; Kates et al., 1962). Several studies have shown that supplementation with dietary proteins increases the humoral and cellular responses to infection with other GIN species, including *T. colubriformis* (see Kambara et al., 1993; van Houtert et al., 1995a), *Nematodirus battus* (see Israf et al., 1996) and *Teladorsagia circumcincta* (see Coop et al., 1995).

So far, the evidence from pen studies of tropical breeds of sheep and goats infected with *H. contortus* have not shown clear effects of supplementary protein feeding on host resistance, when measured by the reduction of faecal egg counts (FECs), worm burdens, and female worm length and fertility. In the case of meat producing goats, studies by Blackburn et al. (1991, 1992) did not show any reduction of *H. contortus* egg excretion or worm burdens with an increased plane of nutrition. On the other hand, Nnadi et al. (2007) showed a reduction in FECs in *H. contortus*-infected West African Dwarf goats supplemented with palm kernel cake, and Nnadi et al. (2009) showed a longer pre-patent period of this nematode in supplemented West African Dwarf goats. Another study (Phengvichith and Ledin, 2007) also showed reduced FECs in *H. contortus*-infected Laos goats following an improvement of their plane of nutrition using the plants Gliricidia and Cassava. Pen studies with sheep failed to reveal a clear reduction in FECs in infected Menz and Horro sheep supplemented with cotton seed cake (Haile et al., 2002).

Although the effect of protein supplementation on resistance to GINs is not clear, there is solid and consistent evidence showing that supplementation with rumen-escape protein significantly reduces the pathophysiological effects of haemonchosis and, hence, improves host resilience.

Early studies with *H. contortus* (Abbott et al., 1985a, 1985b, 1986a, 1986b, 1988; Kates et al., 1962, Preston and Allonby, 1979) showed that lambs given a diet with high crude protein showed less severe pathophysiological effects (anaemia evaluated by PCV) and clinical signs (weight loss, feed intake and mortality) than animals on a low-protein diet, despite similar levels of gastroenteric blood loss. Further pen studies with sheep confirmed this improved resilience against *Haemonchus* infection (Bricarello et al., 2005; Haile et al., 2002, 2004; Khan et al., 2012). More detailed studies of *H. contortus*-infeted sheep (Datta et al., 1998; Wallace et al., 1995, 1996) estimated the supply of ME and MP, and found similar results to those quoted previously, which had used "empirical" differences in protein supply, as defined by Bown et al. (1991). One exception is the work by Rocha et al. (2011), who did not show any improvement in resilience of peri-parturient ewes (Santa Ines and Ile de France) when protein supplementation was given.

Similarly, pen studies involving *H. contortus* infection in meat-producing goats confirmed that a high plane of nutrition resulted in improved resilience (Blackburn et al., 1991; Nnadi et al., 2007, 2009; Phengvichith and Ledin, 2007). For example, the studies by Blackburn et al. (1991, 1992) showed that young goats infected with *H. contortus*, when offered a high-protein diet, had better live-weight gains than those offered a low protein diet, despite a lack of effect on resistance.

Nowadays, on one hand, the use of meat and fish meals, which have been widely used in previous studies, are seriously challenged in developed countries, because of societal issues (e.g., human health issues linked to prion risk). On the other hand, the price of some feedstuffs used previously as rumen-escape proteins is becoming prohibitive for developing countries. This situation has led to the search for local sources of Non-Protein Nitrogen (NPN) that can optimize the production of microbial proteins in the rumen. Hence, urea and/or locally available by-products containing inexpensive rumen-degradable proteins have been investigated.

An initial pen trial showed that the addition of urea to a basal diet reduced the pathophysiological effects of *H. contortus* and improved productivity in susceptible sheep (Wallace et al., 1994, 1998). Similar results were then obtained in lambs trickle-infected with *H. contortus* and *Tr. colubriformis* (see Knox and Steel, 1996). However, the latter experiment used urea-treated oaten chaff that might have suffered delignification, thus increasing the digestibility of cellulose of the chaff, and overcoming N deficiency in the rumen (Van-Soest, 1994). Studies of goats have shown that increasing the level of urea in urea-molasses blocks (UMBs) or giving urea supplements alone gave no production benefits or reduction in parasite burdens or FECs. Only when urea was accompanied by cotton-seed meal was there a beneficial effect of supplementation (Haile et al., 2002; Knox and Steel, 1996; Singh et al., 1995). The lack of response to NPN suggests that the rumen degradable protein supply from the basal diet was not limiting and that the supplemented NPN was wasted via urine (Van-Soest, 1994). As previously mentioned, the sole use of NPN might not have been sufficient to promote optimal ruminal function. The latter function can be achieved by adding a source of rumen degradable carbohydrates that favour the utilization of non-protein nitrogen (NPN) sources to improve microbial efficiency (Van-Soest, 1994). The lack of synthesis of specific amino acids might have also affected animal responses (Van-Houtert and Sykes, 1996).

Based on the hypotheses that requirements for host immune response or some production parameters can depend on specific amino acids (e.g., sulfur-containing amino acids for wool production) (Mac Rae, 1993) and on their impacts on amino acid digestion in the infected small intestine, a range of studies have evaluated diet supplementation with specific amino acids, which were protected from ruminal degradation, on host resilience and resistance:

Most of the pathophysiological studies of amino acids have concerned leucine and methionine and have been performed in rodent models (cf. Sakkas et al., 2012b). Moreover, *H. contortus* was rarely the GIN model in pen studies with ruminants. Yu et al. (1998) examined the effects of *T. colubriformis* infection on the metabolism of leucine in the small intestine. Coop et al. (1998) showed that the supplementation of lambs with protected methionine improved the rate of live-weight gain and wool growth, and reduced the adverse effects of *T. colubriformis* infection when compared to un-supplemented pair-fed controls. Increased amino acids in the diet have also been associated with lower *Tel. circumcincta* burdens when compared with non-supplemented lambs (Richardson et al., 1998). However, contradictory evidence exists and indicates that protein or methionine supplementation possibly failed to influence the rate of resistance development to *Tel. circumcincta* infection (Coop et al., 1997). The lack of significant results was attributed to a high protein level in both experimental diets. On the other hand, the addition of protected methionine to the diet did not improve the resilience or resistance of dairy goats to *T.*

colubriformis, either as a single infection (Chartier et al., 1998) or in a mixed infection with *H. contortus* (see Bouquet et al., 1997).

These studies, conducted under confined conditions, allowed the identification of several host factors that influence the interactions between host and nutrition and, consequently, the efficiency of manipulating the host nutritional status, in terms of resistance and resilience. These factors should be considered when interpreting the results from supplementation studies aimed at favouring both aspects of host response to GIN infections.

- ***Age of animals***:

Effects on host resistance (and related immunological mechanisms) and resilience are strongly influenced by the age of animals (Abbott and Holmes, 1990; Kambara et al., 1993). In particular, the initial establishment of nematodes in young, non-immune animals did not seem to be affected by dietary proteins (Coop et al., 1995; Van-Houtert and Sykes, 1996). Therefore, Coop and Kyriazakis (1999) suggested that host nutrition does not affect the rate of the *acquisition of immunity* to nematode parasites. However, it is evident that host protein and energy nutrition can affect the *expression of immunity* in wool- and meat-producing sheep at the latter stages of growth in young and adult animals.

- ***Reproduction***:

In livestock ruminants, host requirements are at the highest level during pregnancy and lactation. As far as GIN infections are concerned, the time around parturition is usually described as the "periparturient relaxation of immunity" (PPRI), which is linked to significant increases in FECs in both ewes (e.g., Donaldson et al., 1998; Leyva et al., 1982) and does (e.g., Chartier et al., 1998; Rahman and Collins, 1992) compared with non-pregnant female animals. This PPRI has been attributed to a "breakdown" in host immune response under such physiological circumstances (Barger, 1993; Leyva et al., 1982). Apart from the hypothesis that connects several reproductive hormones (e.g., prolactin, progesterone/oestrogen) and the host immune system, there is a second hypothesis that proposes that the PPRI is related to the nutritional stress around the time of parturition (Barger, 1993; Coop and Kyriazakis, 1999). Based on the second hypothesis, it is predicted that supplementation would assist in alleviating the PPRI (i.e. restoring a degree of immunity) and the consequences of infection (resilience) around parturition.

PPRI has been confirmed in ewes or does experimentally infected with *H. contortus* (see Macarthur et al., 2013a, 2013b; O'Sullivan and Donald, 1973; Valderrabano et al., 2005), and in natural infections under tropical conditions (Costa, 1983; Gonzalez-Garduño et al., 2014; Rocha et al., 2004). Because of the importance of PPRI in the epidemiology of GIN (Barger, 1993; Donaldson et al. 1998), nutritional manipulation around parturition (including protein flushing) appears to be a sustainable method of managing infections in offspring with less reliance on synthetic chemical anthelmintics (Houdijk et al., 2012). It is worth noting also that the majority of studies of nutrition - GIN infection interactions in reproductive ewes or does, whether under experimental or natural infection conditions, involved either species of *Teladorsagia* (e.g. Houdijk et al., 2003, 2005) or *Trichostrongylus* (e.g. Etter et al., 1999, 2000; Kahn et al., 2003a, 2003b) or both parasites (Donaldson et al., 1998), rather than *H. contortus.*

In addition, some rodent models of GIN infections (e.g., *Nippostrongylus brasiliensis* in rats) have also been used to elucidate underlying immunological mechanisms (Jones et al., 2011; Sakkas et al., 2011, Sakkas et al., 2012a, 2012b). However, regarding the interactions between the host nutritional status and GIN infections, it is important to note that interpretations of results from rodent (monogastric) models may not apply directly to (polygastric) ruminants. Most of the early studies focused on the possible effect of improved host nutrition by supplementation with metabolizable protein (Etter et al., 1999; Houdijk et al., 2003, 2005; Kahn et al., 2003). Thereafter, numerous results, mostly from GIN models other than *H. contortus*, have stressed the complexity of the interactions between digestive parasitism and host nutrition, because:

(a) overall, in ruminants, despite the "scarce resource theory" and associated framework (see Sub-section 2.2), it is difficult to separate protein and energy metabolism, because, in ruminal processes, strong interactions occur between host protein and energy metabolism (see Sub-section 2.4);

(b) as far as the previous point is concerned, it has been shown that the nutritional status and fat reserves of ewes *prior to parturition* are key factors, which can influence the PPRI. Using the *H. contortus* model of infection, Valderrabano et al. (2005) showed that an overall higher nutritional level of supplementation during the early *ante partum* period contributed to modulating fat reserves in ewes and positively influenced host immune response during parturition, as measured by FEC, worm numbers and female length and fertility. More recently, a series of studies in Australia investigated the interactions between protein supplementation and fat score in ewes during pregnancy, with a focus on host resistance and resilience (Macarthur et al., 2013a, 2013b). The conclusions were that fat and protein reserves of reproductive ewes during pregnancy led to better host resistance and reduced the PPRI (estimated by FEC), particularly before lambing. In contrast, *ante partum* protein supplementation did not influence host resistance. In terms of resilience, both energy status (evaluated by fat score) and protein supplementation have positive effects on twin-bearing ewes and on lamb survival. Moreover, the fat score also contributed to a higher level and better quality (measured by fat and protein content) of milk. These results for *H. contortus* infection confirmed earlier data from studies with other GIN species (Donaldson et al., 1998; Valderrano and Uriarte, 2003).

(c) some recent results that compared xylose-treated soyabean *versus* faba bean in *Teladorsagia*-infected ewes also suggested that protein quality is an important factor to consider (Sakkas et al., 2012a). However, it was not established whether this was due to the amount of specific amino acids or to the presence of bioactive plant secondary metabolites.

- ***The level of animal production***

This represents another important host factor to be considered when exploring interactions between nutrition and host resistance and resilience to GIN (including *H. contortus*) infections. GIN infections in lactating ruminants, particularly in selected dairy breeds of small ruminants, allow the study of (i) the negative effects of worms in the digestive tract on host nutritional status and on milk production, and (ii) the benefits of manipulating host diets. The interest in dairy ewes or goat models is based on the availability of sensitive methods that can estimate effects on host resilience when compared with fibre- and meat-producing sheep or goats. This aspect relates to the possibility of being able to collect individual milk samples at different times during lactation and to explore factors of variation affecting the balance between host requirements and nutritional status within a herd. The regular collection of quantitative (milk yield) and qualitiative (protein and fat composition) data, in parallel with parasitological information, enables an effective evaluation of the GIN effects on host resilience and resistance parameters, and can assist in testing the theoretical framework described in Sub-section 2.2 (Fig. 3).

A series of studies have explored the resistance and resilience of dairy goats, depending on their level of milk production under different conditions of GIN infections. The main results have shown that:

- Within a group of dairy goats with mixed experimental infections with *H. contortus* and *T. colubriformis*, the does with the highest level of milk production were less resistant (had significantly higher FECs) and less resilient (significantly larger reductions in milk yield, changes in milk quality, including fat content) to GIN infections. Moreover, differences were particularly prominent in the first months after kidding (i.e. the peak of lactation), which represents a period of high nutritional stress (Chartier and Hoste, 1997; Hoste and Chartier, 1993, 1998).
- Thereafter, it was found that a monthly scheme of anthelmintic treatment in a dairy goat herd, under natural infection conditions, led to significantly improved production responses, in terms of milk yield by goats with high production potential, compared with the low-producing does (Chartier and Hoste, 1994).

Dairy goats provide a useful model for measuring the response to protein supplementation for improving resilience and resistance in GIN infected ruminants (Chartier et al., 2000). Under conditions of natural infections in a dairy goat herd, an improvement of both resistance (lower FECs) and resilience (higher milk yield and fat contents) was expressed when does received a diet that covered 125% of the protein requirements *versus* 106% in controls.

Overall, these results confirmed that there was an inverse relationship between the importance of milk yield of does (related either to individual production or to stage of lactation) and the response (resilience and resistance status) to GIN infections, most of which included *H. contortus*. However, it is difficult to dissect the nutritional *versus* the genetic components in such studies. The same comment applies when considering the effects of protein and other nutritional supplementations on resilience and resistance of genetically susceptible and resistant breeds.

- ***Genetic factors***

The general framework suggests that highly productive sheep or goat breeds will be particularly susceptible to a combination of *Haemonchus* infection and poor nutritional conditions. Conversely, highly productive breeds should benefit most from any nutritional improvement. Challenges with *H. contortus* were used in early studies to compare effects of protein supplementation on resilience and/or resistance of two sheep breeds that differed in their susceptibility to GIN infections. In those studies, responses to improved planes of nutrition were reported for Scottish Blackface lambs (the genetically "resistant" breed) when compared with susceptible Finn Dorset (Abbott et al., 1985a, 1985b) or Hampshire lambs (Wallace et al., 1995, 1996). In both studies, high protein nutritional planes were linked to improved resilience in both breeds. An improvement in resistance (measured by significant decreases in FECs) was only found in the "susceptible" breeds.

Controversial results were obtained in a series of studies conducted in Brazil (Amarante, 2014; Bricarello et al., 2005; Rocha et al., 2004, 2011), which examined the interactions between nutrition and genotype in local (Santa Ines) and imported (Ile de France) sheep breeds. On one hand, it was shown that protein supplementation (mainly from soyabean meal) resulted in significantly lower worm burdens (i.e. improved resistance) in Santa Ines lambs, but not in the Ile de France lambs (more susceptible and more productive breed). On the other hand, there was no effect of protein supplementation on fecal egg output of either breed (Rocha et al., 2004, 2011). Indeed, supplementary feeding might not be the best tool to regulate the worm population *per se.*

2.3.2. Evaluating the role of supplementary feeding in grazing animals

The study of the effect of supplementary feeding on resilience and resistance against GINs is more complex under field conditions than under controlled pen conditions. However, field studies could be more relevant in practical terms, as they represent the real situation. However, supplementing grazing animals may reduce their pasture intake, and, consequently, their nematode larval intake. This substitution could confound the interpretation of the effects of supplementation on resilience or resistance against GIN (Van-Houtert and Sykes, 1996). However, a certain level of substitution between pasture and supplement can be considered a positive benefit of supplementary feeding (Retama-Flores et al., 2012).

A first series of studies that assessed the link between dietary supplementation and the degree of nematode control on production responses in grazing lambs were performed nearly 20 years ago (Shaw et al., 1995; Van-Houtert et al., 1995a, 1996). In those studies, supplementation (including energy sources) during parasite infection enhanced the resilience of grazing sheep to GINs. Improved resilience achieved with oatmeal as a low-protein supplement (Van-Houtert et al., 1996) constitutes evidence that energy supplements might have a positive effect on the total supply of metabolisable energy and/or proteins to the host. This finding is important because energy supplements are normally cheaper than those based on protein meals (Van-Houtert et al., 1996). More recently, field studies have been performed in tropical conditions, involving goats and sheep. In all cases it was possible to use supplementary feeding to improve resilience against mixed GIN infections, measured as improved bodyweight gain and diminished pathophysiological effects from infection, as illustrated for Criollo kids fed a combined sorghum/soybean supplement during the wet season (Gutierrez-Segura et al., 2003; Martínez-Ortíz de Montellano et al., 2007; Torres-Acosta et al., 2004) and the dry season (Torres-Acosta et al., 2006). Only one study evaluated the role of UMBs (Waruiru et al., 2004), showing that supplemented small East African goats improved bodyweight gain compared with non-supplemented animals. The same positive results on resilience were reported in the wet and dry seasons for Santa Ines sheep given supplementary soybean meal (Louvandini et al., 2006), and Menz and Menz x Awassi lambs fed a concentrated feed (Tibbo et al., 2008). Meanwhile, it was shown that young Fiji ewes benefited from UMBs, achieving higher weight at mating and positive effects on birth rate and post-natal survival, compared with un-supplemented ewes (Knox and Steel, 1996; Knox, 2003).

It is evident that supplementary feeding, in general, is a good management option that may benefit naturally infected sheep and goats. However, it is important to define when and for how long supplementation is required for grazing animals. Although Datta et al. (1998) reported that short periods of enhanced post-weaning nutrition might have long-term and perhaps life-long effects on production in sheep infected with *H. contortus,* other studies of growing goats in Mexico showed that the long-term effects of supplementation might be too difficult to maintain beyond the period of supplementation (Torres-Acosta, 2006). For instance, Aguilar-Caballero et al. (2002) showed that growing kids receiving supplementary feeding (100 g/day of sorghum:soybean meal 70:30) during the dry season showed a marked improvement on production and health, but this positive effect was not maintained if the supplement was suppressed during the wet season, when forage was abundant. Similarly, growing goats are far more resilient when they receive supplementary feed in the wet season, but this positive effect is not carried-over to the following dry season if the supplement is not maintained. Thus, it seems that growing animals cannot accumulate nutrients, which might be useful in relation to nutritional restrictions, even during the rainy season.

So far, there is only one published field study (Faye et al., 2003) that evaluated the role of supplementary feeding (cotton-seed + rice bran) on adult goats, showing improved milk production and less pathophysiological effects compared with non-supplemented goats. Of all the field studies referred to in this Sub-section, only a few showed signs of improved resistance against GINs. In goats, Gutierrez-Segura et al. (2003) showed a reduction in the excretion of GIN eggs and reduction of fecundity and female length, and Waruiru et al. (2004) reported a reduction in FECs and worm burdens. In sheep, only Louvandini et al. (2006) reported a reduction in FECs and worm burdens in supplemented animals compared with non-supplemented controls. In conclusion, the few field trials of sheep and goats showed that resilience against GIN infections can be increased by supplementation with less degradable (rumen-escape) protein, as was expected from pen studies. The use of UMBs has also produced encouraging results, but they were related to higher VFI. However, the results of supplementary feeding on resistance are less clear. In addition, it is important to indicate that the effects of supplementation on the pathophysiology of infection in grazing calves have been poorly evaluated, and findings have not been conclusive (Magaya et al., 1997).

2.4. Supplementation with dietary energy

It would be inappropriate to study protein metabolism without considering energy metabolism. Protein and energy interact because dietary proteins are a source of dietary energy; energy is needed for rumen function, protein turnover and deposition, because deposited protein represents part of the body’s energy stores. In sheep, when protein supply is adequate, body fat can be used as a source of energy to support protein supplementation when exogenous energy is lacking. Under these circumstances, changes in exogenous energy supply have little or no effect on protein metabolism, provided the endogenous store of energy (body fat) is adequate (Chowdhury et al., 1995). Thus, the use of dietary protein to improve resilience and resistance against GINs may be more effective in sheep than in goats, because sheep are better able to obtain energy for extra N retention by mobilizing their more abundant fat reserves. Meanwhile, leaner carcasses of goats might suggest that insufficient exogenous energy supply might affect their protein metabolism more than that of sheep. For instance, in a pen study with Criollo kids trickle-infected with *H. contortus* and fed iso-energetic diets differing in the level of MP using soybean meal, information was obtained on the pathophysiological effects of infection during a 5-month trial, together with information on diet digestibilities and N balance

(Torres-Acosta, 1999). The results showed that kids had minor pathophysiological effects, irrespective of the nutritional level, compared with non-infected groups, suggesting that the level of infection applied was mild. However, the salient finding of this study was that, although kids on the high protein diet tended to retain more protein than kids on the low protein diet, the former did not achieve a higher growth rate. Moreover, a large proportion of the extra N given to kids was ultimately transformed into urea, and this resulted in greater elimination of N via urine from soybean-supplemented kids.

Under field conditions in the tropical forest of Yucatan, Mexico, several studies have demonstrated that browsing sheep and goats eat a large quantity of N-rich legumes (Ortega-Reyes et al., 1985; Rios and Riley, 1985). More recent studies showed that sheep and goats eat up to 150% of their metabolizable protein requirements (Gonzalez-Pech et al., 2015). Even though some authors concluded that dietary energy was of no use in improving resilience or resistance against, it was evident that, under the conditions of the tropical forest of Yucatán, the best option was to supplement the animals with a source or rumen fermentable energy (RFE). The approach was to optimize the use of available protein rich fodder from tropical forest vegetation to produce more microbial protein and more volatile fatty acids to improve animal performance (resilience) and possibly increase resistance to GIN infections. The first attempt was performed by Gutierrez-Segura et al. (2003), who compared non-supplemented kids *versus* kids supplemented either with maize (108 g/day) or a combination of maize:soybean (70:30%; 107 g/day) during the rainy season. In this study, both supplementation strategies significantly improved resilience compared with non-supplemented control kids. Another field study compared two sources of RFE - maize (107g/day) *versus* sugar cane molasses (150 g/day) - in growing kids during the rainy season, showing that both sources of energy, which represented the same amount of RFE, enhanced resilience to GIN infection(s) compared with non-supplemented control animals (Landa-Cansigno et al., unpublished data). Subsequent studies determined the optimal quantity of RFE to supplement either goats or sheep, by providing the supplement as a proportion of the body weight of animals rather than giving a set amount of supplement daily, as in previous studies (Landa-Cansigno et al., unpublished data). The proposal was to further optimize rumen function by providing the quantity of energy required to achieve optimum animal productivity, and then evaluate the effect of this strategy on a natural parasitic infection. The first study (Garetero-Gallardo et al.,2015) evaluated three naturally infected groups: infected not supplemented (I-NS), infected + maize supplement at 108 g/d (I-S108), maize supplement at 1% of bodyweight (I-S1%), maize supplement at 1.5% body weight (I-S1.5%), or infected + supplemented (maize supplement 1.5% body weight) + moxidectin (0.2 mg/kg body weight subcutaneously every 28 d) (T-S1.5%). Kids browsed daily (7 h) in a tropical forest for 112 days during the rainy season and were weighed weekly to adjust supplementary feeding. Haematocrit

(PCV), haemoglobin (Hb), and FECs were determined fortnightly. On day 112, five kids per group were slaughtered to determine worm burdens. Kids from the I-S1.5% group showed similar body-weight change, PCV and haemoglobin, compared with kids without GINs (T-S1.5%), while showing FECs and *Tr. colubriformis* worm burden compared with the I-NS group ($P < 0.05$). Thus, among the supplement levels tested, maize supplementation at 1.5% body weight of kids was the best strategy to improve their resilience and resistance against natural GIN under tropical rain forest conditions. In the field study of sheep, the treatment groups included the I-NS (infected, not supplemented), I-S (infected, supplemented with maize at 1.5% bodyweight), T-NS (treated with moxidectin 0.2 mg/kg body weight every 28 days, and not supplemented) and T-S (treated with moxidectin and supplemented with maize at 1.5% bodyweight). Again, maize supplementation helped to improve the resilience of hair sheep lambs against GIN infections. The I-S and T-NS groups showed similar live weight gain, haemoglobin and PCV ($P > 0.05$), and both were higher than the I-NS group ($P < 0.05$). Supplemented groups (T-S and I-S) showed higher total DMI (fodder + maize; $P < 0.05$), and, hence, higher intakes of metabolisable energy and proteins than non-supplemented groups (T-NS and I-NS). In both studies, it was possible to establish the cost of natural mixed parasitism due to the simultaneous presence of animal groups with and without infection, and also with and without supplements. Thus, under similar conditions of mixed natural GIN infections and supplementation levels in the tropical forest during the rainy season, Retama-Flores et al. (2012) estimated the average metabolic cost of GIN infections to be 43.5 g body weight/day in Pelibuey lambs browsing in the semi-deciduous tropical forest. This value is similar to the differences recorded in kids in the study by Gárate Gallardo et al. (2015), with 41 g body weight/day between infected/non-supplemented and infected/supplemented groups.

2.5. Supplementation with mineral micronutrients and trace elements

There is evidence to suggest that also mineral micronutrients and trace elements are involved in the interactions between host nutrition and GIN infections:

(1) Mineral metabolism can be seriously affected in the presence of worms (Knox et al., 2006; Mc Clure, 2008). Sykes and Greer (2003) suggested that few studies have shown that phosphorus, calcium, copper and magnesium metabolism are disturbed by presence of GINs, and that further studies are necessary to assess the role of this aspects. In haemonchosis, iron metabolism can be strongly modified, with increased haemoglobin and blood turnover in the host. Studies of lambs showed that small intestinal infections with *T. vitrinus* had an effect on phosphorus absorption/metabolism, leading to clinical signs (Coop and Field, 1983).

(2) Some of these micronutrients (copper, molybdate, cobalt, zinc, selenium and vitamin E) are important for proper immune function (Bundy and Golden, 1987; Mc Clure, 2003, 2008; Suttle and Jones, 1988).

(3) Some micronutrients (i.e. copper) are important for nematode biology, because they act as co-enzymes for various key processes in worm metabolism (digestive proteinases of the worm) (Suttle et al., 1992).

However, to date, very few studies and results obtained have led to the manipulation of mineral and trace elements to improve *H. contortus* control, and more generally, GIN infections. This is explained by (**i)** the complexity of physiological interactions with mineral nutrition (e.g., interactions between copper and molybdate metabolism) in the host**; (ii)** the lack of basic information and understanding of the interactions between mineral and parasitic nematodes in the infected host, and **(iii**) the role of external factors, such as soil conditions (i.e. whether or not there is a deficiency in a given trace element in feed resources). Such environmental conditions also govern the decision whether to add trace elements to the diet, because of their potential toxic effect on the hosts (i.e. copper toxicity in sheep and, to a lesser extent, in goats).

One main exception is the manipulation of copper through the use of Copper Oxide Wire Particles (COWPs). This particular material has a long lasting effect, as COWPs dissolve gradually in the abomasum of treated animals, releasing copper over the course of several weeks. COWPs were initially commercialized to correct copper deficiency in ruminant production systems. Some earlier results showed that the use of COWPs could contribute to lower GIN populations in sheep (Bang et al., 1990) and goats (Chartier et al., 2000). Several further studies in both small ruminant species have also demonstrated a primary effect against abomasal species, particularly *H. contortus* (see Burke et al., 2007, 2010; Soli et al., 2010). The role of COWPs is first to prevent the establishment of incoming third-stage infective larvae, and, second, to cure established infections, as suggested by Knox (2002). A recent efficacy trial showed that COWPs have a significant effect for up to 35 days against *H. contortus* in sheep (Galindo-Barboza et al., 2012).

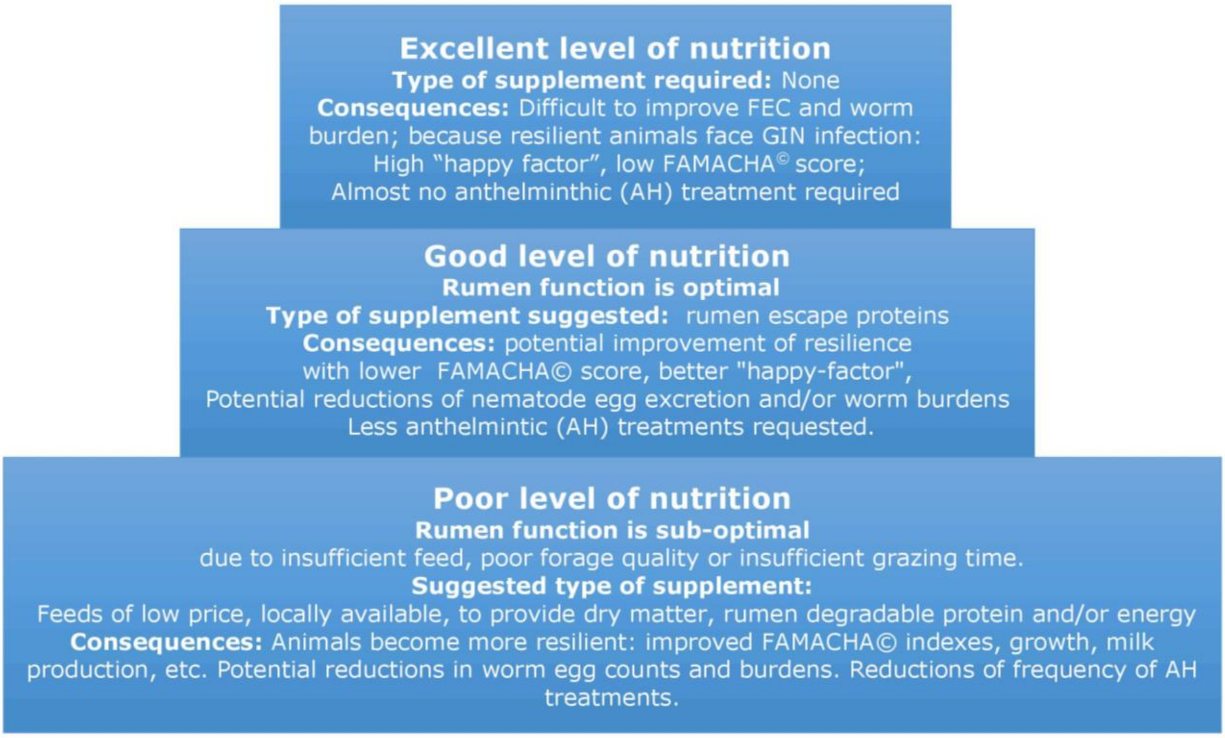
Due to the high prevalence of *H. contortus* in tropical and subtropical areas, various studies have focused on examining the use of COWPs under a wide range of conditions (Burke et al., 2004, 2007; Luginbuhl et al., 2006; Martínez-Ortiz-de-Montellano et al., 2007). Overall, the data have shown that COWPs can be a valuable alternative to anthelmintic drugs for control of *H. contortus,* either alone, or in combination with other nutritional options (Martínez-Ortiz-de-Montellano et al., 2007). For instance, Burke et al. (2005) reported the complementarity of use of COWPs and *Duddingtonia flagrans* (nematode-trapping fungus) for *H. contortus* control in lambs. The question of potential cumulative hepatic toxicity of copper has been addressed in sheep (Burke et al., 2007) and goats, suggesting a lower risk in the latter species (Galindo-Barboza et al., 2012; Martínez-Ortiz-de-Montellano et al., 2007).

2.6. A scheme to improve the control of *H. contortus* infection on the farm, depending on the nutritional status

If nutrient manipulation is to be used in the control of *H. contortus* (and other GINs), the following nutritional principles should be considered under farm conditions (Fig. 4):

2.6.1. Animals on a poor nutritional level

It is evident that animals with poor nutritional status, such as the majority of animals under resource-poor farming conditions, may benefit greatly from supplementary feeding. The most important target would be to provide nutrients that are essential to improve and optimize ruminal function. Providing the appropriate supplement is key. On farms where there is insufficient feed, animals should receive a source of forage with at least 8% crude proteins (i.e. grass hay or crop stubbles). If the latter contains < 8% crude protein, it will be necessary to include a source of NPN (Leng, 1991), such as poultry litter. But, as mentioned previously, the improvement in host resilience with NPN might not be achieved, unless it is coupled simultaneously to a source of rumen degradable energy (i.e., sugar cane molasses). Similarly, for malnourished animals browsing legume-rich foliage, as is the case in the tropical forests of Mexico (González-Pech et al., 2015), animals may need a source of rumen degradable energy to increase the utilization of digestable protein from the foliage harvested. Thus, it is evident that for most farmers, the decision to improve the nutritional status of their animals is complex. That is why computer decision support software, such as "Grazfeed" (Freer et al., 1997), has been created, but such software packages are often not applicable under many conditions, as they require basic nutritional information that may not be available for many ecosystems, which differ from temperate grasslands. To avoid the complexity of nutritional management decisions, many farmers are forced to buy expensive feeds composed of conventional feedstuffs, such as maize, sorghum or soybean. These feeds already include the main macronutrients (protein, energy, fibre) necessary to supplement ruminants, but are costly and also compete with the needs of humans.



Excellent level of nutrition
Type of supplement required: None
Consequences: Difficult to improve FEC and worm burden; because resilient animals face GIN infection: High "happy factor", low FAMACHA© score; Almost no anthelminthic (AH) treatment required

Good level of nutrition
Rumen function is optimal
Type of supplement suggested: rumen escape proteins
Consequences: potential improvement of resilience with lower FAMACHA© score, better "happy-factor", Potential reductions of nematode egg excretion and/or worm burdens Less anthelmintic (AH) treatments requested.

Poor level of nutrition
Rumen function is sub-optimal
due to insufficient feed, poor forage quality or insufficient grazing time.
Suggested type of supplement:
Feeds of low price, locally available, to provide dry matter, rumen degradable protein and/or energy
Consequences: Animals become more resilient: improved FAMACHA© indexes, growth, milk production, etc. Potential reductions in worm egg counts and burdens. Reductions of frequency of AH treatments.

Figure 4: The outcome of supplementary feeding for the control of *Haemonchus contortus* and other gastrointestinal nematodes is dependent on the level of animal nutrition. At the lowest nutritional level, which is the most common situation in many farming systems, there are more chances to improve health and productivity, while improvements will be less evident in animals with a good level of nutrition and there might be very limited improvement in those few animals, which have an excellent level of nutrition. "Happy factor" correspond to factors avoiding to use chemical drugs to control GIN infection (Kenyon and Jackson, 2012)

2.6.2. Animals on a good nutritional level

In the case of farming systems, where animals graze excellent quality grasses, and where the amount of grass and grazing time is sufficient to maintain a good level of nutrition, animals should have optimal ruminal function. Thus, any further improvement in nutrition can only be achieved with a source of dietary protein in the form of rumen-escape protein (e.g., fish meal, soybean meal), which is expensive; any attempt to use such feed for ruminants should be based on a clear cost-benefit calculation.

2.6.3. Animals on an excellent nutritional level

For animals with an excellent level of nutrition, dietary supplementation will not further improve the nutritional status. Thus, the only viable intervention would be to reduce the cost of the diet without reducing its quality, and this would be more a matter for nutritionists and economists. Under such conditions, there might not be a direct improvement in the control of GINs with supplementary feeding, except during the periparturient period or lactation. Supplementary feeding will be coupled to very good farming conditions, where GIN burdens would be mild or very low.

3. QUALITATIVE ASPECTS

Over the last 20 years, an increasing number of studies have been focused on exploring and validating a second aspect of the interactions between host nutrition and GIN infections, that is whether the composition or quality of the feeds/plants eaten by ruminants can affect worm biology.

Overall, the results suggest that some natural products, also called plant secondary metabolites (PSMs) (see Box 1), which are either already present in or can be added to ruminant diets, could have anthelmintic effects on nematodes (Hoste et al., 2006, 2012; Min et al., 2003). The presence of these secondary metabolites is thought to explain the antiparasitic activities of herbal remedies and/or nutraceuticals (Andlauer and Fürst, 2002).

Due to their mode of application and objective, i.e. short-term use for treating the animals, herbal remedies (phyto-therapeutic drugs) are somewhat related to pharmacological, synthetic AH drugs. In contrast, as indicated by the name, the "nutraceutical concept", which is based on a combination of "nutrition" and "pharmaceutical", is closely linked to host nutrition. In veterinary helminthology, a nutraceutical can be defined as a livestock feed, which provides both nutritional value and a health benefit to animals, and is used to prevent and control diseases. The differences between nutraceuticals, phytotherapeutic drugs and synthetic drugs have been thoroughly discussed in a recent review (Hoste et al., 2015). Because this chapter covers the interaction between host nutrition and *Haemonchus* infection, Section 3 will focus on the interactions between nutraceuticals, the biology of *H. contortus* and the consequences of haemonchosis in the host. We will not consider here any data on the AH effects of phytotherapeutic drugs.

Bioactive plants or plant-based resources containing condensed tannins and other polyphenols are currently receiving considerable attention and, hence, represent interesting nutraceutical models for: (i) studying the *in vivo* AH effects in infected sheep and goats, (ii) examining the potential mechanisms of action of a group of PSMs against GINs and (iii) exploring potential on farm-applications, which will depend on regional epidemiological and environmental conditions, as far as different tannin-containing resources and different GINs are concerned. Chicory (*Cichorium intybus*) is another interesting example of a plant that has been explored as a nutraceutical. In this case, sesquiterpene lactones, not tannins, are thought to be the main bioactive PSMs (Foster et al., 2011a, 2011b; Molan et al., 2003b). However, under the climatic and agronomic conditions favourable to growing chicory, *Teladorsagia, Ostertagia* or *Trichostrongylus* spp. are the dominant GINs. Therefore, the potential AH effects of chicory against *H. contortus* have rarely been investigated (e.g., Athanasiadou et al., 2007; Marley et al., 2006; Tzamaloukas et al., 2006). In a study of lambs that compared two tannin-containing legumes with chicory, the total reductions of *H. contortus* FECs were 63% for sainfoin and *Lotus corniculatus* and 89% for chicory. However, a tendency towards lower *Haemonchus* worm numbers was noticed with sainfoin (-35%) and birdsfoot trefoil (- 49%), but not for the chicory diet (Heckendorn et al., 2007).

3.1. Chemistry of tannins and related polyphenols.

The plant kingdom contains an amazing array of secondary metabolites on which herbal medicine has been founded for centuries. The plant metabolites (i.e. PSMs) range from being highly bioactive and potentially highly toxic and contribute to nutraceutical effects of dietary feeds (Andlauer and Fürst, 2002). Here, we focus on one group of important nutraceuticals, i.e. tannins and related polyphenolic compounds. These compounds are also well known for their anti-oxidant properties (Barbehenn et al., 2006*).* Figure 5 illustrates the best-known tannins from terrestrial plants (condensed tannins, gallotannins and ellagitannins), their monomeric building blocks (including flavan-3-ols, gallic acid and ellagic acid) and flavonoids, which are biosynthetically related polyphenols (see also Figure 6). Several reviews exist on the analysis of tannins (Huemmer and Schreier, 2008; Mueller-Harvey, 2001; Salminen and Karonen, 2011; Schofield et al., 2001), and efforts continue to improve their detection and analysis (Engström et al., 2014; Gea et al., 2011; Grabber et al., 2013; Zeller et al., 2015a), and to study the relationships between their chemical structures and biological effects.

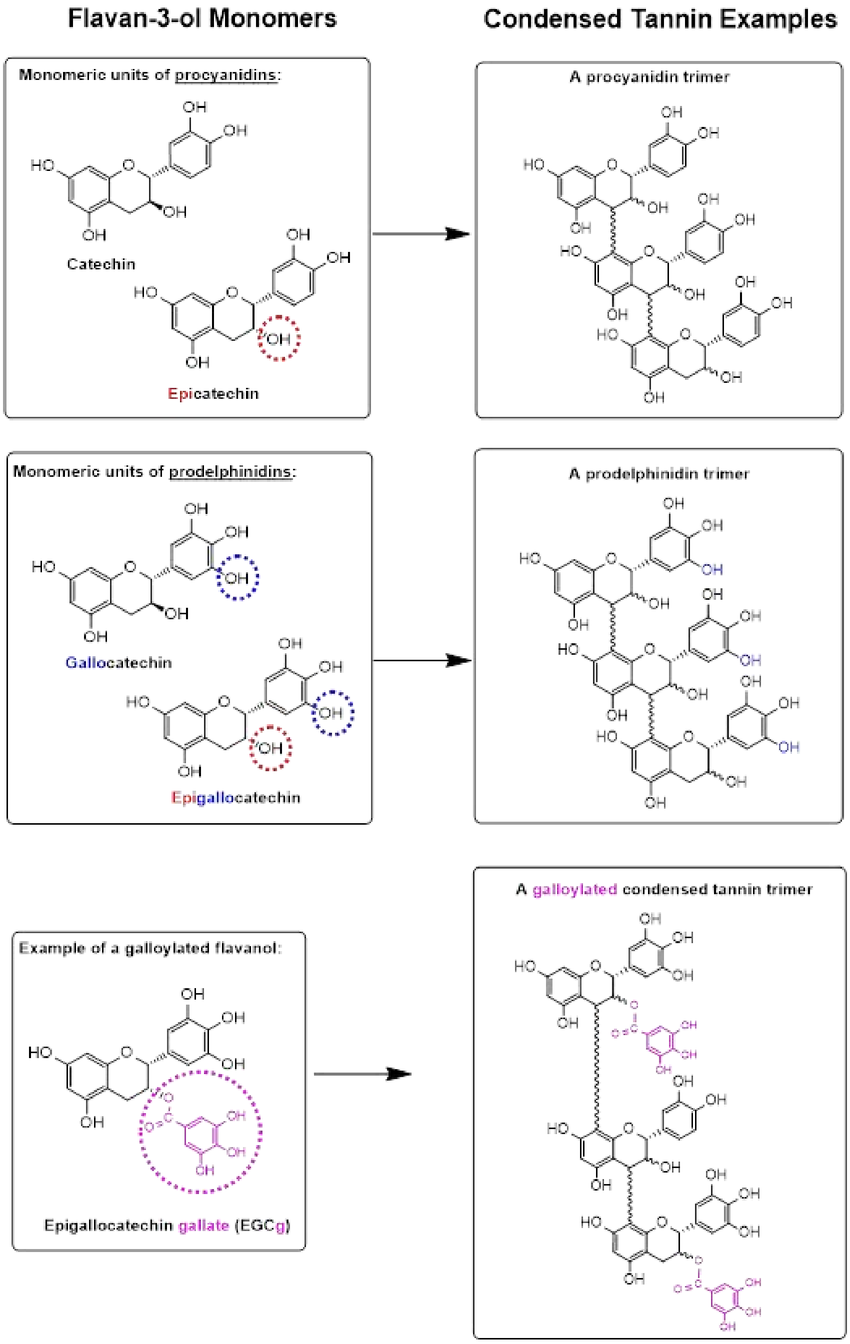


Figure 5: Flavonoids and tannins with anthelmintic effects against *Haemonchus contortus*

- Monomeric flavonoids: flavan-3-ols, other flavonoids (quercetin, luteolin, naringenin, etc) (widespread in plants)
- Condensed tannins (forage legumes, browse, etc)
- Galloylated derivatives of CT (*carob, A. nilotica*)
- Tannic acid
- Taratannins (*P. lentiscus*)

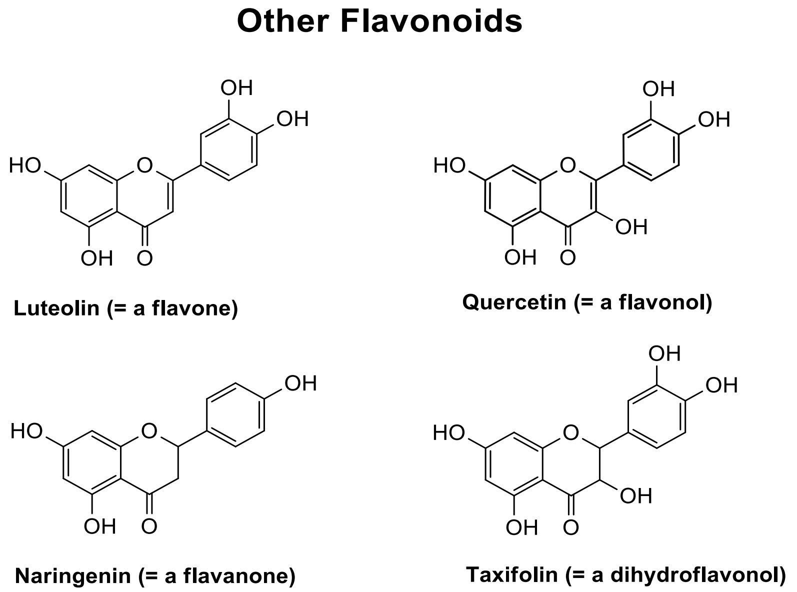


Figure 6: Chemical structures of other anthelmintic flavonoids

3.2 Methodological issues

3.2.1. Analytical tools for tannins

The complexity of plant polyphenol chemistry demands information on the advantages and disadvantages of the analytical tools being used to assess their biological, anthelmintic, effects. Much research has been undertaken using non-specific colorimetric assays, which can neither distinguish between compounds nor different compound classes (Mueller-Harvey, 2001; Salminen and Karonen, 2011; Schofield et al., 2001). Currently, there are only a few relatively simple methods that provide some useful information on tannins. The HCl-butanol assay is specific for condensed tannins (Grabber et al., 2013), whereas polyethylene glycol (PEG) and polyvinyl pyrrolidone (PVPP) are 'tannin-neutralising' compounds. The latter two methods are widely used for *in vitro* and *in vivo* studies (Brunet et al., 2008; Silanikove et al., 2001). However, PEG or PVPP do not distinguish between condensed or hydrolysable tannins, and they are not always perfect at 'neutralising' tannins, as they can also bind other polyphenols (Azando et al., 2011; Barrau et al., 2005). Tannin analysis is now benefitting from rapid advances in instrumentation, i.e. ultra performance liquid chromatography-tandem mass spectrometric (UPLC/MS/MS), nuclear magnetic resonance and other techniques (Engström et al., 2014; Mancilla-Leytón et al., 2014; Zeller et al., 2015a).

3.2.2. Studying the anthelmintic properties of tannins and related polyphenols

The different approaches that can be used to evaluate and interpret the AH effects of bioactive compound and tannin-containing nutraceuticals have advantages and disadvantages, and have been reviewed recently (Hoste et al., 2015). In essence, a four-step approach is considered suitable for **(i)** the initial identification of likely bioactive plants; **(ii)** subsequent *in vitro* screening; **(iii)** confirmation under controlled *in vivo* conditions and **(iv)** validation under farm and epidemiological conditions to develop practical applications.

H. contortus has been widely used as a GIN model in these studies, because of **(i)** the apparent expansion of prevalence of this GIN under various epidemiological conditions; **(ii)** the basic information that is now available on the genetics and genomics of *H. contortus*, which includes isolates that are resistant to synthetic AHs and **(iii)** the severity of the pathological/ pathophysiological consequences in individual sheep and goats and related economic costs at the whole-herd level; mortalities due to haemonchosis are particularly serious on small farms in the tropics.

3.3. Impact on the biology of different key stages of gastrointestinal nematodes

Most results have been obtained from a whole range of *in vitro* assays (Jackson and Hoste, 2010) (Table 1) that were applied to different developmental stages of *H. contortus* (Table 2). They have been followed up with *in vivo* studies of *H. contortus*-infected small ruminants under either confined (Table 3) or natural conditions (Table 4). Here, we refer to *in vivo* studies, where the results relate to specific effects on *H. contortus*, either because it was the sole nematode or because adapted methodologies have been applied to identify its presence and/or its specific consequences. This chapter describes findings for sheep or goats from temperate or tropical regions.

Studies of infected animals fed with tannin-containing plants have shown that three main stages of *H. contortus* are targets:

(1) ***Infective third-stage larvae (L3)****. In vivo* results have demonstrated lower L3 establishment rates in hosts that consume selected bioactive resources.

(2) ***Adult worms*****.** Most studies of small ruminants that examined the effects of CT-containing plants on adult nematode populations found that there was a significantly lower overall FEC in animals infected with *H. contortus*. This observation/finding relates either to a lower worm numbers and/or lower fertility female *H. contortus*.

(3) ***Eggs****.* There are numerous *in vitro* studies that used egg hatch and/or larval development assays with *H. contortus*. Overall, these studies suggest impaired egg hatching and/or development in the presence of CT. Some *in vivo* results showed impaired development of *H. contortus* egg to the L3 stage *in vitro* (e.g., Shaik et al., 2006). However, other data were less consistent, with discrepancies in significance between *in vitro* and *in vivo* results.

3.4. Impact on host resilience

Besides the effects on *H. contortus* biology, multiple studies have repeatedly demonstrated that tannin-containing nutraceuticals can have positive effects on host resilience in sheep and goats. These investigations were conducted in the context of controlled experimental infections or in situations where *Haemonchus* was the dominant species. These beneficial effects included: (i) better production parameters, in particular body weight gain in lambs, (ii) less severe signs of anaemia in goats and sheep, and (iii) lower mortality rates when challenged with parasites. Some studies also recorded the number of AH treatments as an index of resilience required to control haemonchosis. All references that describe PSM effects on the worm life cycle or resilience are listed in Tables 2 to 4 (cf. Sub-section 3.7).

It is difficult to separate the effects of tannins on worm biology (e.g., reduction in worm populations or female worm fecundity) from the beneficial effects on host pathophysiology. Moreover, apart from tannin content, the protein content and rumen-escape protein effects, which occur in the presence of some tannin types (Waghorn, 2008), can also be important factors. Hence, the mechanisms for improved resilience are still unclear and worthy of future exploration.

3.5. Variability in effects of plant secondary metabolites depending on parasitic nematodes

In comparison to synthetic AH molecules, tanniniferous nutraceuticals do not result in a 99 % elimination of GINs from hosts. These nutraceuticals exert their effects mainly through combined impacts on GIN biology by interfering with the three key life cycle stages (eggs, L3 and/or adult worms). The net result is to slow down the dynamics of GIN infection and to maintain infection at levels that are compatible with small ruminant production and farm economy. Consumption of CT-containing plants leads to lower environmental contamination (Min et al, 2005) or host infection (Brunet et al., 2007) - but it does not usually eliminate worm populations from hosts. In addition, nutraceuticals and the PSM products, unlike synthetic AH drugs, are not used in pure and standardised forms. Hence, several factors can impact on their activity (Figure 7), and these are key issues to consider for future farm applications.

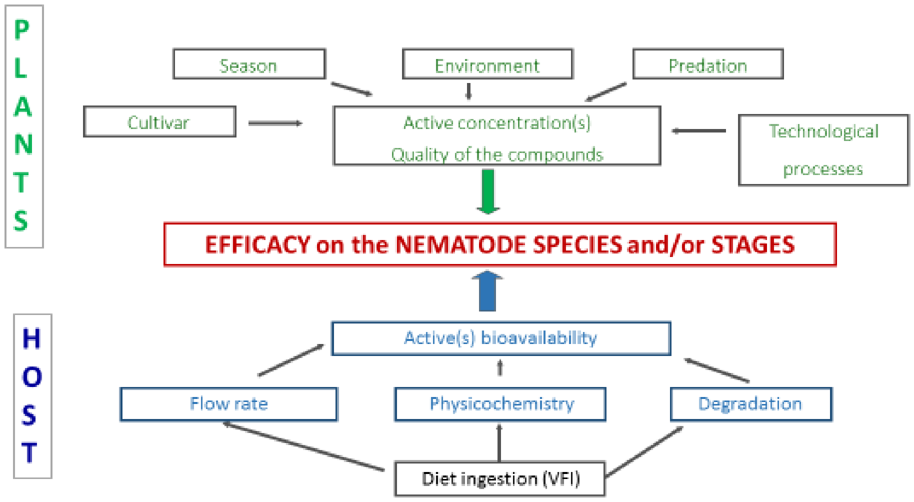


Figure 7: A summary of the main factors which might explain the variability in the efficacy of nutraceuticals against *H. contortus* based on the example of tannin containing resources.

3.5.1. Variations arising from nematode species and life cycle stages

Results from a wide range of studies conducted on CT-containing plants suggest that PSM effects on nematode biology appear to depend on the parasite species. This aspect was first illustrated in studies of quebracho tannins. When established adult *Haemonchus* populations in sheep were exposed to condensed tannins, lower worm fecundity and worm numbers were found for the intestinal species (*Nematodirus battus* and *T. colubriformis*), but there were no changes in the species that resided in the abomasum (*Te. circumcincta* and *H. contortus*) (Athanasiadou et al., 2001). However, in goats, the effects on adult worms were restricted to lower female fecundity in both *T. colubriformis* and *H. contortus*, but not for *Tel. circumcincta* (see Paolini et al., 2003a, 2003b). On the other hand, the establishment of infective larvae was reduced by nearly 70% for *Te. circumcincta* and *T. colubriformis*, but only by 35% for *H. contortus* (see Paolini et al., 2003a, 2005a). Since these early discoveries, results of *in vivo* studies, under controlled or natural infections, raised various questions. Tables 3 and 4 summarise results from the different studies, which included *H. contortus*, in order to examine whether this species is particularly susceptible to tannins.

It would be reasonable to assume that intrinsic GIN species factors (e. g. differences in protein composition of sheath, cuticle or digestive tract) may explain differences in AH susceptibilities to tannins. If this were the case, a comparison of results from *in vitro* assays (Table 2) against different stages and nematode species should indicate which species is/are most affected by tannins, and these results should also be consistent. However, there is no clear trend (Table 2). Alternatively, the differences in AH effects obtained from *in vivo* studies might also result from differences in bioavailability, as the composition of free and bound tannins varies along the digestive tract. It is hypothesized that free tannins may have a more potent AH activity, although this has not yet been tested. The formation of CT–protein complexes depends on tannin and protein structures, but also on the compositions of tannin solutions. Of particular relevance is the pH of a solution, as it affects the stability of the tannin-protein complexes. It is, therefore, hypothesised that the different ruminal, abomasal and intestinal pH values may modulate tannin bioavailability. This hypothesis needs to be tested. Unfortunately, there are few studies examining the tannin "pharmacology" along the gut (Terrill et al., 1994). These aspects need to be further addressed. In addition, AH effects of tannins and polyphenols can be difficult to explain because their concentration and composition adds another dimension of complexity.

3.5.2 Variation in plant tannin composition (quantity and quality) in relation to activities against **H. contortus.**

Pioneering research in New-Zealand (Min et al., 2003) first discovered that the prodelphinidin tannins in *Lotus pedunculatus* had higher AH activities than the procyanidin tannins in *L. corniculatus* (Figure 5). However, subsequent progress was slow due to the complexity of plant tannins and limitations of analytical techniques. Non-specific assays, i.e. colorimetric or anti-oxidant assays for polyphenols, failed to explain the AH or other biological activities of tannins (Katiki et al., 2013; Salminen and Karonen, 2011).

Several factors influence the composition of bioactive compounds in plants, with plant species, but also varieties or accessions being the most important determinants of tannins and polyphenols (Falchero et al., 2011; Kotze et al., 2009; Lowther et al., 1987; Mueller-Harvey, 2006; Stringano et al., 2012). Knowing the source of such variation will be extremely useful, because it will enable plant breeders to develop new varieties with optimised tannin traits in the future. In many plants, the composition also varies between different plant parts, e.g. sainfoin stems have more procyanidins and sainfoin leaves have more prodelphinidins (Malisch et al., submitted; Theodoridou et al., 2010). Given the large phenotypic variation that typically exists within germplasm collections, it will, therefore, be possible to enhance desirable tannin types (Falchero et al., 2011; Hayot Carbonero et al., 2011), when the most important traits relating to AH activity are known.

Additional factors are growth stage and environment (Azuhnwi et al., 2013a; Grabber et al., 2014; Kotze et al., 2009; Theodoridou et al., 2011). Fortunately, there is also some evidence that tannins and polyphenols are affected by genotype-environment interactions and that some accessions have particularly 'robust' compositions, which are less subject to environment influences (Azuhnwi et al., 2013b; Kotze et al., 2009). Future research will need to focus on identifying plant varieties that can deliver consistent AH properties (Luescher et al., 2014).

The processing of harvested plants via drying (hay), pelleting or ensiling can also impact polyphenol composition. Pelleting and ensiling lead to a higher proportion of bound tannins that are less easily extracted using solvents (Lorenz et al., 2011; Minnée et al., 2002; Terrill et al., 2007). Whilst both processes do not affect the AH properties of *L. cuneata*, others appear to significantly improve AH effects following the ensiling of sainfoin (Manolaraki, 2011; Ojeda-Robertos et al., 2010). These differences also require verification in future studies.

Leaves and stems of sericea lespedeza (SL) were analysed for CT concentration and composition, with higher CT levels found in leaves than stems (16.0 g and 3.3 g/100 g dry weight, respectively). The CT of both leaves and stems were almost pure prodelphinidins (98% and 94%, respectively), while the CT polymer size was much larger in SL leaves than stems (mean degree of polymerization: 42 and 18, respectively). It has been suggested that the excellent AH effects of SL may stem from its unusual CT composition; the higher proportion of prodelphinidins than procyanidins and high molecular weights have both been linked to better anti-parasitic bioactivity (Novobilský et al., 2013).

3.6. Modes of action of tannin-containing plants against *H. contortus*: direct *versus* indirect effects

The number of studies that explore how tannins and other polyphenols (flavonoids) act against the different key parasitic GIN stages is expanding. It is expected that these studies will guide the use of CT-containing nutraceuticals under farm conditions in the future. Two general hypotheses have been proposed to explain the AH effects of tannins and other polyphenols against parasitic GINs. The main results to support these two non-exclusive hypotheses are described below.

3.6.1 The direct (= pharmacological-like) hypothesis

The bulk of the *in vitro* results for *H. contortus* tend to support the first hypothesis (see Table 2), because host factors make no contribution. To a lesser extent, short-term *in vivo* studies (Athanasiadou et al., 2001; Brunet et al., 2008b) have also provided some evidence for the direct effects of PSMs against *H. contortus*. In addition, some data are now emerging that tannin structures can impact on *in vitro* and *in vivo* results. This information comes from scanning (SEM) or transmission electron microscopy (TEM) studies, which studied different stages of *H. contortus* (adult worm and/or L3 stages).

For example, structural and ultrastructural changes were induced in adult female *H. contortus* after *in vitro* and/or *in vivo* contact with CT-containig legumes, such as tzalam (*Lysiloma latisiliquum*), sainfoin *(O. viciifolia*) or quebracho (*Schinopsis spp*) extracts; none of these changes were detected in control worms. The most evident changes detected in treated worms by SEM were on/in the cuticle and the buccal capsule, and provided striking evidence of shrivelled larvae/worms and of blocked orifices that probably disabled the worm from feeding, and decreased fecundity (Hoste et al., 2012; Martinez-Ortiz de Montellano et al., 2013; Williams et al., 2014b). Similarly, in goats fed with sericea lespedeza (*Lespedeza cuneata*), all worms had deep cuticular ridges and a shrunken, disheveled appearance (Gujja et al., 2013; Kommuru et al., 2015). Using methods of transmission electron microscopy, the main lesions and alterations detected were in the hypodermis, and lesions were also observed in intestinal and muscular cells of ensheathed and exsheated infective L3 (Brunet et al., 2011) and adult worms (Martinez-Ortiz de Montellano et al., 2015). Taken together, the results of these different *in vivo* and *in vitro* studies provided evidence of direct interactions between various CT-containing resources and L3 or adult *H. contortus*. It is hypothetised that the phenotypic changes in worms following exposure to CT-containing plants relate to reduced motility, nutritional uptake and reproduction (Hoste et al., 2012). It is likely that the strong propensity of tannins towards proline-rich proteins accounts for these observations. Tannins possess numerous polyphenolic groups that confer 'stickiness' as they facilitate a plethora of hydrogen bonds, and their benzene rings give rise to hydrophobic interactions. Together, these charateristics lead to strong interactions in protein-tannin complexes (Dobreva et al., 2011; Kilmister et al., 2015). Further testing of the "direct" hypothesis will require a better understanding of the pharmacology of condensed tannins and polyphenols along the gut, in particular on the interactions of tannins and proteins in the different digestive organs (Rochfort et al., 2008). Work in this direction has commenced in sheep and cattle (Desrues et al., 2015; Quijada et al., 2015).

3.6.2. The indirect (= immune-based) hypothesis

Condensed tannins can also have an indirect effect on nematode biology by improving the host's immune response due to their ability to bind with proteins of the digestive content. The successive processes are as follows: (i) formation of tannin-protein complexes in the rumen, which decrease ruminal protein degradation; (ii) then, an increased flow of rumen-escape proteins into the abomasum and increased absorption of amino acids and peptides in the small intestine; and (iii) consequently, a better nutrition of the host. Based on the concepts described in Section 2 of this chapter, this succession of events can explain the improved host immune response against GINs, including *H. contortus* (Hoste et al., 2012). Recently, Tedeschi et al. (2014) reviewed in detail the currently available information of the impact of condensed tannins on immune responses.

Moreover, *in vitro* studies have shown, that tannins have immune-modulating effects. Condensed tannins can prime both human and ruminant γδ T cells *in vitro* (Holderness et al., 2007; Tibe et al., 2012). Interestingly, tannic acid, which is a mixture of gallotannins, has been shown to modulate the immune responses of sheep, which were stimulated by *H. contortus* antigen, by inhibiting Th1 cytokines and by increasing Th2 cytokine expression *in vitro* in white blood cells (Zhong et al., 2014).

- *In vivo* study of circulating immune potent cells have confirmed these results *in vitro*. An increased number of γδ T-cells in sheep fed with CT-containing *Salix* was also reported (Ramírez-Restrepo et al., 2010). More recently, according to Singh et al. (2015), the humoral and cell mediated immune responses are higher in animals receiving feed containing condensed tannins (a mixture of *Eugenia jambolana/Psidium guajava*), and lower *H. contortus* FECs were noticed in goats.
- In the case of *H. contortus* infection, only a few *in vivo* studies have been completed by pathological examinations that have tested this "indirect" hypothesis. These experiments compared the numbers of different effectors cells (eosinophils, mast cells, globule leucocytes and goblet cells) against GINs (Balic et al., 2000) in the mucosae of the digestive tract of sheep (Martínez-Ortíz-De-Montellano et al., 2010; Rios de Alvarez et al., 2010) or goats (Paolini et al., 2003 a, 2003b) which had been given a CT resource or control diet. The most convincing evidence of an enhanced local/mucosal immune response showed that when lambs grazed on sulla or chicory, they had a reduced development of worms and significantly higher numbers of mast cells and globule leucocytes in the abomasal mucosa. However, *Tel. circimcincta* was the main parasitic species involved (Tzamaloukas et al. 2006). In other studies involving *H. contortus* infection, results were less convincing. These include studies of sheep fed the CT-containing *L. latisilliquum* browse (Martinez Ortiz de Montellano et al., 2010) or goats fed quebracho (Paolini et al., 2003c) or *sericea lespedeza* diets (Joshi et al., 2011). Usually, insignificant differences in the number of effector cells in the digestive mucosae of small ruminants were measured in response to CT-containing diets. From these studies, the authors concluded that sericea lespedeza and other CT-containing legumes had little effect on the local host immune responses, and that the observed differences were mainly due to the direct action of CT on *H. contortus*.

3.6.3. Emerging information on structure-activity relationships, and possible mechanisms of action

Most forage legumes possess procyanidin (PC)-prodelphinidin (PD) mixtures (Figure 6), which are extremely difficult to separate, which precludes structure-activity relationships (SAR) studies (Stringano et al., 2012). Furthermore, we have noticed certain trends in the biosynthesis of tannins: many plants tend to produce smaller PCs and larger PDs, and high tannin contents are often associated with higher percentages of PD tannins – although there are some exceptions. Therefore, these associations can confound SAR studies. However, a few plants are biosynthetic specialists, and by isolating their tannins, it is possible to investigate features that contribute most to AH activity. Several studies have now explored AH differences between small *versus* large tannins, different PC/PD and *cis/trans*-flavanol ratios, between A- and B-type inter-flavanol linkages and effects of galloylated tannins (Figure 6; Mueller-Harvey, 2006).

Thus far, the conclusions are that higher AH activity against many different GINs, including *H. contortus*, is found in condensed tannins that have:

- A high proportion of prodelphinidins rather than procyanidins (Desrues et al., 2016; Novobiliský et al., 2013; Quijada et al., 2015; Williams et al., 2014a,);
- Larger rather than smaller tannins (Desrues et al., 2016; Novobiliský et al., 2013; Quijada et al., 2015,).
- Stereochemical differences arising from *cis- versus trans*-flavanol units in tannins do not appear to be important, although it must be pointed out that only a few plants have tannins, which are composed largely of *trans*-flavanols in, for example, the leaves from black currant (*Ribes nigrum*) and a few *Salix* species. This statement limits the range of tannins that has been tested so far.

Possible explanations for these observations are that:

- Prodelphinidins have more phenolic groups and are thus able to form more hydrogen bonds with proteins than procyanidins;
- Larger tannins are better able to crosslink different proteins, as shown by precipitation studies (Kilmister et al., 2016; Zeller et al., 2015 b).

It would be interesting to explore whether some of the above features accounted for the fact that GIN exposed to tzalam (*L. latisiliquum*) or sainfoin (*O. viciifolia*) revealed quite different features of structural damage in electron microscopic images (Martínez-Ortíz-de-Montellano et al., 2013).

More research will be needed also on the AH effects of galloylated tannins (i.e. condensed tannins substituted with gallic acid and also gallotannins). *In vitro* studies (Brunet and Hoste, 2006; Molan and Faraj, 2010; Ramsay et al., 2015) have clearly shown that the presence of gallic acid esters enhanced the AH activity of flavanol monomers and condensed tannins. Tannic acid, which consists of gallotannins (Figure 6), disturbed the larval mobility as assessed using an *in vitro* test (Zhong et al., 2014). However, neither carob pods nor *Acacia nilotica* leaves (Table 5) reduced *H. contortus* FECs, or worm numbers or fecundity (Arroyo-Lopez et al., 2014; Kahiya et al., 2003). As far as we are aware, these are the only AH trials conducted thus far using feed containing highly galloylated tannins - although possibly of low molecular weights. Therefore, other plants with galloylated, condensed tannins or with gallotannins, such as *Rumex* sp., grapeseeds, persimmon and *Camellia sinensis*, might be worth studying (Li et al., 2010; Ropiak et al., submitted; Salminen et al., 1999; Spencer et al., 2007; Tuominen et al., 2013). As only a small number of studies (Dahmoune et al., 2014; Mancilla-Leyton et al., 2014; Rodriguez-Perez et al., 2013; Romani et al., 2002) have reported extent of galloylation in condensed tannins or quantified gallotannins, it is possible that galloylation effects may have been overlooked in the past. We emphasize this point, because of the exciting results obtained recently by feeding goats and sheep on *Pistacia lentiscus* (see Azaizeh et al., 2013; Landau et al., 2010; Manolaraki et al., 2010), which contains low molecular weight gallate esters (i.e. galloylated sugars and taratannins) and condensed tannins (Dahmoune et al., 2014; Mancilla-Leyton et al., 2014; Rodriguez-Perez et al., 2013; Romani et al., 2002).

According to Fakae et al. (2000), substituted phenol-based anthelmintics inhibit helminth glutathione S-transferases, which appear to be important for establishing chronic infections. Therefore, in addition to tannins, there are several other polyphenolic compounds that have demonstrated AH activity against *H. contortus* (see Kotze et al., 2009). These compounds include flavones (luteolin, zapotin, 5, 6, 2′-trimethoxyflavone, 5,6,2′,5′,6′-pentamethoxy-3′,4′-methylenedioxyflavone), flavonols (quercetin; Barrau et al., 2005) and a flavanone (luteolin; Klongsiriwet et al., 2015). Given that all green plants contain a wide range of monomeric flavonoids, it is of interest that synergistic effects were observed between condensed tannins and luteolin or quercetin on L3s (Klongsiriwet et al., 2015). In contrast, some negative interactions between tannins and other PSMs have been evoked to explain inconsistent results obtained with some extracts on *H. contortus* eggs in a hatch assay (Vargas-Magaña et al., 2014a).

Recently, Kumarasingha et al. (2014) reported that combinations of plant extracts against *C. elegans*, a free-living nematode, used as a model for *H. contortus* and other GINs because of accumulated and extensive knowledge of its biology and genome, elicited stress responses that differed compared with those induced by synthetic AH drugs. This interesting result will need evaluation on *H. contortus*.

Table 5 is a first attempt to link information from disparate studies on CT-containing plants with *in vitro* and *in vivo* activities against *H. contortus*. This table provides key information on analytical methods [e.g., condensed tannins by thiolysis or HCl-butanol assays; hydrolysable tannins by high performance liquid chromatography (HPLC) analysis of gallic and ellagic acids; general tannin methods by polyethylene gycol (PEG) or polyvinylpolypyrrolidone (PVPP) addition] and on the various parameters that were assessed on *H. contortus*. Table 5 includes forage legumes, browse plants and agro-industrial by-products, although many other browse plants and herbal drugs also represent interesting sources of condensed tannins (Ropiak et al., submitted).

Table 6 shows the tannin composition of selected tree leaves and browse plants; the procyanidin/prodelphinidin ratios range from 100/0 to 7/93; tannin sizes, as measured by the mean degree of polymerisation from 4 to 34, and condensed tannins content of 3 to 17% of dry matter. However, Table 5 does not contain information on the AH activity against *H. contortus* for three widely studied plants (*L. pedunculatus, Dorycnium rectum* and *Rumex obtusifolius*) with demonstrated efficacy against other GINs. Such AH effects are also expected against *H. contortus*, but still need to be tested.

Structure-activity studies have shown that larger tannins and also high proportions of prodelphinidins are linked to high AH efficacy. The presence of galloylated flavanols and tannins enhances *in vitro* activity, which may account for the excellent results observed using *Pistacia lentiscus*. However, further research will need to ascertain optimum tannin contents, polymer sizes and percentages of prodelphinidin and galloylated tannins, in order to (i) minimise any anti-nutritional effects from such feeds, (ii) assess what dose is required and (iii) explain the results from unsuccessful feeding trials with plant materials that were expected to yield good AH effects (e.g., carob pods and *Acacia nilotica* leaves).

3.7. Towards the on-farm use of condensed tannins (CT)-containing nutraceuticals as anthelmintics

The first empirical results obtained in New Zealand reported significant reduction of FECs in sheep when grazing pastures with a range of different tannin-containing legumes, i.e. birdsfoot trefoil and big trefoil (*Lotus corniculatus, L. pedunculatus*), sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychis viciifolia*) (Niezen et al., 1995, 1998, 2002). These findings provided some directions for future research. However, *H. contortus* was not assessed in these studies. These early results have given a strong impetus to seek AH properties in a wide diversity of plants, characterised by the common presence of significant concentrations of CTs and polyphenols. The wide distribution of tannins and polyphenols in the Plant Kingdom makes this a worthy endeavour. The main botanical resources investigated to date include:

(i) Legume forages (Fabaceae) suited to large-scale agronomy;
(ii) tropical browse resources, which have enabled the exploration of a diversity of forages and leguminous trees , and the complexity of interactions between PSMs as well as between the small ruminants and the local environment; and
(iii) agro-industrial by-products.

3.7.1 Temperate legume forages: sericea lespedeza and sainfoin

In Europe and USA, this recent surge of interest has focused on two legume species of the subfamily Faboideae, as potential nutraceutical forages. These two plants are the Chinese bush clover also known as sericea lespedeza (SL) (*Lespedeza cuneata*)] in the USA and South Africa (Joshi et al., 2011; Kommuru et al., 2014; Shaik et al., 2006; Terrill et al., 2009; 2012; http://www.acsrpc.org/) and sainfoin (*Onobrychis viciifoliae*) in Europe (Heckendorn et al., 2006; 2007; Manolaraki et al., 2010; Paolini et al., 2003c, 2005b; http://sainfoin.eu; www.legumeplus.eu).

The particular focus on these two legumes is explained by: **(a)** their ability to produce seeds, to be grown (cultivated) efficiently, and to organise their production for farm application on a large scale (see below), and also **(b)** the fact that many studies with these two plants have targeted *H. contortus* infections in both sheep and goats. Some other tannin containing legumes have been mentioned (e.g., birdsfoot and big trefoils (*Lotus corniculatus, L. pedunculatus*), sulla (*Hedysarum coronarium*) (see Tables 2 and 3) as potential nutraceuticals against GINs. However, in most cases, *Teladorsagia* and *Trichostrongylus* spp. were the dominant nematodes (Marley et al., 2006; Niezen et al., 1995, 1998). There has been limited *in vivo* study of the effect of *L. corniculatus* or sulla on *H. contortus* (Heckendorn et al., 2007).

Sericea lespedeza [SL; *Lespedeza cuneata* (Dum-Cours.) G.Don.] is a tannin-containing warm-season perennial legume that is well-adapted to the eastern and southern USA and other parts of the world, including Asia, Australia and southern Africa (Mosjidis and Terrill, 2013). It has been an important forage and soil conservation crop in the USA for > 100 years, because of a number of useful agronomic properties, including drought resistance due to a deep root system, tolerance of acidic soils high in free AL^{+3} levels, low fertilization requirements (ability to produce its own nitrogen and mine phosphorus from the soil profile), and limited susceptibility to insects and disease (Hoveland et al., 1990). In contrast, it is also worth noting that some non-native species exploited for these properties are considered by environmentalists and ecologists to be invasive.

Sainfoin (*O. viciifolia)* belongs to the family Fabaceae, the tribe Hedysareae. The genus *Onobrychis* represents > 170 species. Two main botanical forms are identified as *O. viciifoliae*: **(1)** the single cut (common type), which is slow establishing and is providing one flowering and one cut per year and **(2**) the double cut (giant type), which is faster growing and able to provide two to three cuts yearly. Sainfoin is cultivated in temperate climatic zones and used as a perennial legume forage. It is well adapted to basic (chalk) soil and tolerates drought, cold and low nutrients in soil. In Europe, sainfoin was well known until the 1950.

Thereafter, its decline was related to the lower yield and persistence compared with lucerne (*Medicago sativa*) and different clovers (*Trifolium*), which have benefited from genetic selection (Azunhwi, 2012). Because of different advantages to ruminant nutrition and health, and for environmental issues (see reviews by Mueller-Harvey, 2006; Rochfort et al., 2008; Waghorn, 2008), sainfoin has been "rediscovered" in the last 20 years (http://sainfoin.eu; http://www.legumeplus.eu). Its benefits are **(i)** a reduced requirement for chemical fertilisers because of biological nitrogen fixation; (**ii**) high palatability for ruminants and feeding values; **(iii)** positive effects by reducing ruminal methane emission and green house gases**; (iv)** anti bloat effects; and **(v)** a switch of nitrogen excretion from urine to faeces.

Results obtained on sericea lespedeza and sainfoin have provided a bulk of information to address some key issues regarding AH properties related to on farm applications. The data obtained for these two models of legume forage nutraceuticals, which relate to applied objectives, have provided useful information on other types of resources (tropical browse or by poducts).

Exploiting tannin-containing legumes with AH properties

A) Direct grazing: The most obvious way of exploiting tannin-containing legumes with AH properties is to allow small ruminants to directly graze or browse on pastures. This approach corresponds to the early studies of various GINs (except *H. contortus*) in New Zealand (Niezen et al., 1995, 1998)*.* In the rest of this section, we review studies conducted under grazing conditions, where *H. contortus* was the most prevalent GIN species. These investigations concerned mainly Sericea lespedeza (SL) (*L. cuneata)*, and studies of direct grazing on sainfoin (*O. viciifolia)* have been far less frequent (Valderrabano et al., 2010).

Studies aiming at comparing the anthelmintic effects when small ruminants graze on SL as a CT-containing legume forage *versus* control (non-tannin-containing) forages have been perfomed either in infected kids and does (Luginbuhl et al., 2013; Min et al., 2004; 2005) and/or in lambs and ewes (Burke et al., 2012a, 2012b). The different non-tanniniferous plants used as control belong to different plant species [i.e. rye/crab grass (*Secale cereal*e/*Digitaria sanguinalis*)] (Min et al., 2004*);* Bermuda grass *(Cynodon dactylon*) (BG) (Mechineni et al., 2014) or tall fescue (*Festuca arundinacea*) (Burke et al., 2012a, 2012b; Min et al., 2005)], adapted to different regions of the USA.

The first investigation of the anti-parasitic properties of SL came from a cross-over design study for two 15-day periods in goats grazing on SL or a control forage (Min et al., 2004). This early study reported a reduction in FECs by more than 70 % (2500 *versus* 710 eggs per g) in the SL *versus* control pastures. Moreover, the SL-fed goats had a lower percentage of GIN eggs developing into L3s in faecal cultures compared with goats grazing on grass pastures (58% *versus* 99%, respectively). These first results confirmed the diversity of impact of a tannin-containing legume on key developmental stages of GINs (see Sub-section 3.3).

Further studies explored different modes of grazing of SL either **(i)** by including ***rotation*** between SL and a control forage in a series of experiments, with does and/or kids (Lughingbul et al., 2013; Min et al., 2005), or sheep and lambs (Burke et al., 2012a,b) or **(ii)** by comparing pure SL pasture *versus* a control *versus* a mixed pasture combining pure SL with a control plant, again in kids and lambs (Burke et al 2012a,b; Luginbuhl et al., 2013). Results of these studies helped to confirm the effects of a CT-containing nutraceutical on the nematode biology, the dynamics of infection and the host responses (summarised in Sub-section 3.3):

- Significant reductions (> 70 %) in FEC or total faecal output were shown. For example, the mean FEC for does grazing on pure SL and on rotation were significantly lower than for the control animals (145, 329 and 894 eggs/g, respectively), while FECs for the SL kids were lower than for the rotation and the control groups (550, 2757 and 3600 eggs per g, respectively) (Min et al., 2005). Similar data and FEC reductions were recorded in a second study, with reduction values reaching almost 90% in kids grazing on SL compared with controls (Luginbuhl et al., 2013). Mechineni et al. (2014) also reported reduced FECs in kids grazing SL or SL mixed with control plant pastures compared with Bermuda control-grass only (95.4% and 71.5% reduction, respectively) by the end of an eight-week trial. Such differences in FECs have been associated with significant decreases in *H. contortus* numbers determined following slaughter (Luginbuhl et al., 2013). However, in many studies, the worm counts did not differ significantly from controls. Data on the fecundity of female *H. contortus* were usually not available. Moreover, a lower larval development from faecal cultures was rarely reported in SL *versus* control animals (Min et al., 2005).
- By using tracer animals, some of these studies also provided information on the possible future infectivity of pastures; hence, an overall effect of the consumption of SL was seen. When grazing on pure SL and in rotation pastures, tracer kids had 78.3% and 61.0% reduced total worm burdens, respectively, compared with those grazing on control pastures, while the numbers of adult *H. contortus*, *Tel. circumcincta*, and *T. colubriformis* were reduced by 89%, 100% and 50%, respectively, in SL-grazed and 78%, 40%, and 50%, respectively, in animals on rotation compared with control tracer kids (Min et al., 2005).
- When estimating the effect of the different systems on the host resilience (see Sub-section 3.1.4), an improved anaemia status was usually reflected in higher PCV values in animals consuming SL, in the rotation or in the mixed (SL+ a control plant) groups, by comparison with control groups (Burke et al., 2012a; Luginbuhl et al., 2013; Min et al., 2005;). However, the effects on live weight gains were usually not significant in a rotation system (Luginbuhl et al., 2013; Mechinenni et al., 2014) or when combining SL and forages not containing tannin (Burke et al., 2012a, 2012b). FInally, one study compared lambs grazing only on SL, SL mixed with tall fescue or Bermuda grass (control). The resilience was evaluated by a reduction in the mean number of required dewormings per lamb during the grazing season. The numbers were 0.71, 0.20, and 0.21 in the Bermuda grass, the mixed pastures and the pure SL groups, respectively (Burke et al., 2012b).

Overall, the conclusions from these grazing studies were that sericea lespedeza, as a model of a tannin-containing legume nutraceutical, represents a viable plant for reducing GIN infections in small ruminants, leading to decreased contaimination of pasture with infective larvae. SL has natural anthelmintic properties, but the effects appear to be primarily due to reduced fecundity of GINs rather than the killing of adult worms. Therefore, producers should exercise caution when taking sheep and goats off SL pastures.

These results also showed that, despite **dilution of tannin-containing legume over time** (by rotation) **or space** (pasture composed with a mixture of SL with a plant not containing tannin/s) (Burke et al., 2012a, 2012b; Luginbuhl et al., 2013), some antiparasitic effects were maintained, although at a lower level than pure SL-grazing (Burke et al., 2012a, 2012b). In grazing studies of goats and sheep, the most consistent anti-parasitic effects were observed in animals on pure SL pastures. When goats were switched between SL and non-SL pastures, FECs rapidly decreased while on SL pastures, and then quickly increased on non-SL pastures, indicating an effect of short-term SL grazing on worm fecundity, rather than the removal of adult GINs. Longer-term exposure to CT from grazed SL reduces the numbers of adult *H. contortus*, *Tel. circumcincta* and, to a lesser extent, *T. colubriformis* (see Min et al., 2005). Grazing on mixed SL grass pasture has demonstrated positive anti-parasitic effects in both goats (Mechineni et al., 2014) and sheep (Burke et al., 2012b), but the effects are often delayed compared with animals on pure SL pastures. This is particularly true for sheep, as they are more reluctant than goats to graze on SL in mixed pastures, but they will graze on SL once they adjust to this plant.

B) Conserved forms (hay, silage and pellets): For on-farm applications, the preservation and subsequent processing of tannin-containing legume forages, such as sericea lespedeza and sainfoin, have several advantages. It gives farmers greater flexibility to use bioactive forages, and facilitates storage and transport to areas where these legumes do not grow well or at all. However, processes such as sun drying, grinding and pelleting can affect tannins, because all processes involving heat are suspected to affect the quantity of condensed tannins (CTs), or alter a plant's CTs from extractable to bound forms.

In a study with sun-dried and fresh-frozen high- and low-tannin sericea lespdeza forage fed to sheep, Terrill et al. (1989) reported that sun-drying decreased the extractable CT content in sericea lespedeza (SL), and improved intake and digestibility of the high-CT forage. Subsequent work (Terrill et al., 1992) showed that CT in dried forages was not reduced, but instead shifted from extractable to bound forms. The effect of processing SL and sainfoin on the bioactivity of CT and related polyphenols is still the subject of on-going research. In this regard, the different conserved forms of tannin-containing resources provide a means: (i) to examine the effects of bioactive plants containing PSMs (CT-containing plants being the model here) under *in vivo* confined conditions against GINs (see Sub-section 3.3) and on the host resilience and/or resistance (Sub-section 3.4); (ii) to better understand the mode of actions of PSMs on worms (Sub-section 3.6).

- *Sainfoin and sericea lespedeza hay:*

Hay processing appears to have little impact on the antiparasitic properties of sun-dried sainfoin or SL compared with fresh material. The first results on the potential use of sainfoin hay were obtained using goats with established (natural) *H. contortus* infection (Paolini et al., 2003c). The authors reported reductions in FECs by 75 % within 3 days following the feeding of sainfoin hay. These reductions persisted for up to 2 weeks after the goats were returned to a (negative) control diet. This persistence of effect suggested a nematocidal effect on worm numbers.

Using SL hay, Shaik et al. (2004) compared isoproteic and isoenergetic diets of ground SL or Bermuda grass hay (80% of the ration) plus 20% concentrates with yearling goats experimentally infected with *H. contortus*. Egg counts were 92% and 86% lower than controls in does given the SL ration on days 21 and 28 of the SL-feeding period, respectively. At the end of the experimental ration period, after all does were put back on to the control ration, the FECs did not significantly differ between groups, suggesting that the main effect related to the fecundity of female *H. contortus*. These results were completed with a follow-up study when naturally infected kids received trickle infection of *H. contortus* L3s, and were then fed unground SL or Bermuda grass hay (75% of the ration) plus concentrates for 7 weeks in pens. FECs in the SL-fed bucks decreased by 80% relative to the control group, seven days after SL feeding was initiated, and these differences increased to 88% by the last 2 weeks of the trial. In addition, the SL-fed goats had reduced abomasal and intestinal GIN numbers. Total reductions in adult female worms were 77%, 36%, and 50% for *H. contortus*, *Tel. circumcincta* and *T. colubriformis*, respectively, in the SL group compared with the controls. The SL-fed group had also a reduced development of infective L3s from GIN eggs from faeces and a higher (average) PCV than the control group (Shaik et al., 2006).

These results were confirmed in lambs for both legume species (SL and Bermuda grass). The effects of feeding unground SL or Bermuda grass hay were compared for experimental lambs trickle infected with *H. contortus* (Lange et al., 2006). Compared with the control group, FECs of the SL-fed lambs decreased by 98% after seven days and remained significantly lower throughout the rest of the trial, with reductions varying from 77% to 86% during the SL-feeding period, and dropping to 37% and 53% after SL was discontinued. Worm counts (limited to three lambs per group) indicated a 67 % reduction of *H. contortus* number in the SL group compared with the control group.

With sainfoin, in lambs experimentally infected with *H. contortus* and *Cooperia curticei* (see Heckendorn et al., 2006), significant reductions in FECs of 58 % and 48 %, respectively, for each of the nematode species were reported. These decreases were associated with a reduction of worm number for *H. contortus*. No significant differences were observed for *C. curticei*.

Hay of tannin-containing legumes has also proved to be efficient at reducing L3 establishment in the host animal. When sainfoin hay was given to goats around experimental infection with *Haemonchus* L3s, a reduction (not statistically significant) of 38 % in worm establishment was reported compared with a control group (Paolini et al., 2005a). In lambs consuming sericea lespedeza hay around the infection period, a 26 % reduction was seen (Lange et al., 2006).

- *Sainfoin silage*

The possibility of exploiting ensiling processes for SL has received little attention. In contrast, several studies have examined the palatability, the nutritive and feeding value and the *in vitro* digestion of sainfoin silage in ruminants (Copani et al., 2014; Luscher et al., 2014; Theodoridou et al., 2010; Wang et al., 2015). In regard of the AH effects, some *in vitro* studies have compared extracts from fresh, hay and ensilage by applying the *in vitro* larval exsheathment inhibition assay (LEIA) (cf. Table 1) to *H contortus*. The results revealed a higher *in vitro* AH activity in hay and silage samples compared with fresh sainfoin. For silage, these differences appear to be explained partly by the presence of aglycon molecules of flavonoids, with higher antiparasitic properties than the corresponding glycoside flavonols (Ojeda-Robertos et al., 2010). In experimentally infected lambs, Heckendorn et al (2006) also compared the anthelmintic effect of sainfoin hay compared with silage. They showed that the reduction in FECs and worm counts were comparable (~ 50 % in both cases) with both conserved forms of sainfoin.

- *Dehydrated pellets of sericea lespedeza or sainfoin.*

The challenges of feeding hay and/or silage to livestock include the difficulty of transport and storage, and possible wastage of valuable feed. Pelleting can help at reducing wastage, improving ease of feed transport and storage and deliver stable products. However, some high heating, combined with high pressure, is usually required to produce the dehydrated pellets. Hence, these harsh treatments during the pelleting processes might destroy some tannins and related polyphenols and/or increase the amount of bound CT in plant material (Terrill et al., 1992). These impacts can seriously affect the bioactivity of the PSMs, in particular the AH activity against *H. contortus*.

To determine the effect of pelleting processes on the anti-parasitic efficacy of ground SL whole plant hay, a study was undertaken with naturally infected bucks (Terrill et al., 2007). After grazing on a grass pasture contaminated with GINs, the bucks were moved into pens and fed a basal control diet for a three-week adjustment period. Then, they were assigned for 28 days, to different dietary experimental treatments consisting of 75% hay and 25% commercial diet pellets. The hay treatments were control ground whole-plant SL, and pelleted whole-plant SL; then all the goats were switched back to the control ration for seven days. Both SL hay and pellets significantly reduced FECs compared with control animals by day seven of the trial, and maintained these differences to the end of the study, even after switching back to the control ration for 7 days. The reduction in FECs relative to controls averaged 70% and 54% for goats given the SL pellets and ground SL hay, respectively. A significant effect on the development of egg to L3 was also less for the goats fed SL pellets than the animals fed Bermuda grass hay, the percentage of *H. contortus* L3 recovered being 25.0% and 83.4%, respectively. Moreover, the goats fed SL pellets had significantly higher PCV values and lower numbers of adult *H. contortus* than control goats. By contrast, for the treatment group fed ground SL, the PCV values and wom numbers did not differ significantly from controls. These authors concluded that pelleting SL hay enhanced its AH efficacy against GINs (Terrill et al., 2007).

Similar results began to be obtained from studies with sainfoin pellets. *In vitro* comparisons between hay (dried for two days) and pellets produced from the same primary material suggested that the pelleting process maintains AH activity. Moroever, a series of *in vivo* studies using sainfoin pellets in ration have confirmed the AH effects against either susceptible or AH resistant strain of *H. contortus* (see Gaudin et al., 2015; Girard et al., 2013). The results have shown overall reductions in FECS, from 30 % to > 50 %, depending on the batch of pellets and the tannin content. These studies with conserved forms of either sericea lespedeza or sainfoin have also helped to compare and explain different factors associated with variation in results (see section 3.5), thus providing useful information for future use under farm conditions.

- ***Components and treatment of legume forage***

As there are higher levels of CT in SL leaves than stems (12.5% *versus* 3.3%, respectively; Mechineni et al., 2014), several studies with SL have focused on an evaluation of leaves, either as ground leaf meal, or leaf meal pellets in the diet of sheep and goats, either fed in confinement, or as a supplement to goats grazing on pasture (Terrill et al., 2012). To determine whether there was any difference in efficacy between sun-dried, whole plant SL and leaves alone, a trial was conducted with kids trickle-infected with *H. contortus* L3s (Terrill et al., 2008). After an adjustment period, with all animals given a control diet, kids were fed either ground SL whole plant or leaf meal (25% of the ration) and received a supplement formulated to make the diets isoenergetic / isoproteic. Both diets reduced FECs to pre-trial levels, but FECs of kids on the SL leaf meal diet decreased more rapidly and were significantly lower than those of goats fed the whole plant SL meal. It was concluded that feeding SL leaves only would increase the efficacy of this tanninniferous legume against GINs.

- ***Tannin concentration and dosage***

The above studies raise an important question: what dietary tannin concentration is needed to affect *H. contortus* biology and hence improve the control of infection?

Most results acquired in the different *in vitro* assays (Table 1), when using *H. contortus* as a model (Table 2), have suggested a dose-dependent response in antiparasitic effects for a whole range of various tannin-containing plants. The hypotheses that the AH effects are dose-dependent as well as that a threshold of CT and related polpyphenols in the diet is needed to achieve AH effects have been supported by few *in vivo* studies, targeting either the infective L3s or the adult worms of *H. contortus*.

For instance, a study with sainfoin hay (Brunet et al., 2007) using cannulated sheep examined the effects of different percentages (0 to 100 %) of sainfoin in the ration on the exsheathment of *Haemonchus* L3s. The results showed that levels of 75% and 100% were needed to achieve significant reductions in L3 exsheatment when compared with controls (0%). With SL hay, a confinement feeding study was performed with naturally infected goats (Terrill et al., 2009). The aim was to examine the effects of different percentages of a combination of ground SL (0%, 25%, 50% and 75%) and conversely ground BG (control) in hay diet on *H. contortus* infection. Results on worm biological traits suggest a proportional relationship between the percentage of SL in the hay part of the diet and the effects on the adult *H. contortus* populations (evaluated by FECs) and egg development into L3. In addition, FEC measurements suggested that a threshold level of SL hay in the ration has to be achieved, because repeated significant reductions relative to control only occurred at levels of 50% and 75% SL in the diet. There was no effect on blood PCV or adult worm numbers in the 25% and 50% SL hay groups, but the goats given the 75% SL hay diet tended to have lower PCVs and had 75% fewer *H. contortus* worms relative to control animals.

To provide an answer as to how much tannin is needed for an anthelmintic effect is far more complex because:

i) AH effects depend both on the quantity and the quality of tannins (See section 3.5). Although *L. cuneata*, many browse species and pine bark extract have high tannin concentrations, positive effects have also been recorded with much lower concentrations with other resources (Table 5 and 6). Currently, there is insufficient information to specify what concentrations of which tannin types will deliver AH activity against *H. contortus*, or for that matter any other GIN, due to the fact that feeds are rarely analysed for their quantitave and qualitative tannin compositions.

ii) Besides tannins, the possible AH effects of some flavonoids (flavanols and flavonols) have been supported by some *In vitro* studies (Brunet et al., 2006; Klongsiriwet et al., 2015; Molan et al., 2003a, 2004b) (Table 6). In addition, a few studies suggest that some interactions, either synergistic or antagonistic, can also occur either between polyphenols (Klongsiriwet et al., 2015) or between different PSMs (Burrit and Provenza, 2000; Lyman et al., 2008; Vargas-Magaña et al., 2014a).

iii) The question of "how much?" also relates to the method to measure/ quantify the tannins (see section 3.1.2). In addition, other active metabolites in the feed participate in the AH activity. However, they are usually not directly measured.

- ***A time frame for the anthelmintic effects in infected ruminants consuming tanniniferous nutraceuticals***

Studies of goats have shown that the reductions in EPG occurred within seven days of initiation of grazing SL (Lughinbul et al., 2013; Min et al., 2005). Longer periods (14 to 35 days) were mentioned in 2 lambs trials (Burke et al., 2012a; 2012b) when grazing mixed pastures with sericea lespedeza and tall fescue. However, the animals were reluctant to graze the SL in the mixed SL-Tall fescue pastures. Overall, these data confirm the hypothesis that for any nutraceutical plant to be exploited against GINs of ruminants, several days of consumption are required to reach sufficient concentrations of PSMs in the different organs of the digestive tract and before any antiparasitic effects can be detected.

Some indications on the persistence of the effects have also been obtained in studies of lambs (Burke et al., 2012b), since benefits of SL grazing on FEC disappeared after 14 days once lambs were moved back to BG pastures following SL grazing. These data on the period of efficiency of nutraceuticals need to be compared with results of studies presented below using conserved forms of tannin-containing legumes.

A relationships between the duration of consumption and the concentration of CTs is suggested by the results, and conclusions of one study of kids. It was shown that the FEC reductions were more rapid and significant in kids grazing on SL only, while FEC reductions in the SL-BG kids were not significant compared with control kids (Lughingbul et al., 2013).

- ***When and how?***

As previously mentioned, conserved forms of tannin-containing legume nutraceuticals offer flexibility in use to regulate natural GIN infections in small ruminants. Studies of hay, silage or pellets of sainfoin and/or of sericea lespedeza have illustrated different options.

- *Prolonged distribution*: A study was completed to determine the AH potential of pelleted SL leaf meal given as a constant supplement to kids when grazing grass pasture (predominantly Bermuda grass and bahiagrass = *Paspalum notatum*). The kids were assigned to three similar pastures, and offered either 75% SL leaf meal pellets, 95% SL leaf meal pellets, or a commercial pellet product at 0.91 kg/head/day using an automatic feeder for the 77-day study. FECs were similar in all treatment groups until day 28, after which both SL treatment groups had significantly lower FECs than the control group through to the end of the trial (days 35-77). Total numbers of adult *H. contortus* and *Tel. circumcincta* were lower (94% and 47%, respectively) in goats given the 95% SL leaf meal pellets as a supplement compared with the commercial pellet product. The authors concluded that feeding 95% SL and 75% SL leaf meal pellets as a supplement to goats grazing on grass pasture both reduced effects of GIN infections, with a greater effect linked to the supplemention with 95% SL pellets*.*

- *Repeated distribution for short-term period:* A second option is to distribute tannin-containing nutraceutical for a few days at regular intervals. A study was perfomed with sainfoin hay in naturally infected goats to examine the effects of such scheme on worm populations and host resilience. *H. contortus, Te. circumcincta* and *T. colubriformis* were the main GINs. Goats in the experimental group received sainfoin hay monthly indoors for seven days. The control goats received ryegrass hay. The two diets were isoenergetic/isoproteic. The distribution of sainfoin was associated with: (i) significant reductions of EPG, related to a decrease in worm fertility for the 3 parasite species but no significant changes in worm numbers; (ii) a better host resilience assessed by higher PCV values and the need of salvation AH treatments for 50 % of the animals in the control but not for any goats in the sainfoin group (Paolini et al., 2005).

o *Targeting the peri-parturient relaxation of immunity (PPRI) in infected small ruminants:*

The anthelmintic properties of sainfoin silage were exploited in a study (Werne et al., 2013), aiming at reducing the periparturient rise in FECs, because of the importance of this biological phenomenon in the epidemiology of GINs (including *H. contortus*) (Chartier et al., 1998; Donaldson et al., 1998). GIN-infected ewes received an experimental infection with *H. contortus* L3, one month prior to lambing. The study investigated the use of sainfoin and/or pellets of faba bean (*Vicia faba*), either as single CT resources or in combination. The periparturient ewes in late gestation were fed for 25 days with either (i) a ryegrass-clover forage (control fed = CF); (ii) sainfoin silage (S); (iii) a combined CT-feed consisting of sainfoin forage plus faba bean pellets (SFB); or (iv) faba bean pellets and ryegrass-clover forage (FB). A fifth group composed of ewes in early gestation (EG) was added to determine the dimension of the PPRI by comparison to the groups in late gestation (i-iv). Compared to the CF group, the differences in FECs over the feeding period were −55 % (S), −40% (SB), +8% (B) and −41% (EG). These results illustrated that (a) CT originating from sainfoin can prevent a PPRI effect with possible consequences on GIN epidemiology; (b) the variability in effect depends on the CT resources, since the faba beans feed did not produce any FEC reduction.

3.7.2. Dissecting the complexity of tropical legumes as nutraceuticals against H. contortus and other gastro intestinal nematodes

Compared to legume nutraceuticals from temperate regions, a small amount of data has been acquired for tannin containing plant materials from the tropical areas of the world. If we consider the biodiversity of plants that may contain bioactive PSMs in the different tropical ecosystems such as humid or subhumid tropical forests, deserts, costal dunes, etc., it is evident that the work performed so far can be considered very limited. It is only in the last 10 -15 years that tropical plants started to be screened for its AH activity (Alonso-Díaz et al., 2010b). The first *in vitro* screening studies evaluated the AH effect of seed extracts against *H. contortus* including *Mangifera indica* l. (Costa et al., 2002) and *Leucaena leucocephala* (Ademola et al., 2005, 2006). Later on, several *in vitro* screening studies aimed at exploring candidates for nutraceutical purposes against GIN infections using extracts obtained from leafs of tannin rich legumes of the tropical forest. The first candidates explored were obtained from several browsing plants commonly consumed by sheep and goats and readily available for farmers: *Acacia pennatula, Acacia gaumerii, Brosimum alicastrum, Havardia albicans, L. leucocephala, Lysiloma latisiliquum* and *Piscidia piscipula* (Alonso-Díaz et al., 2008a, 2008b; Hernández-Orduño et al., 2008; Calderón-Quintal et al., 2010). These studies showed that most plants had a promising AH effect against *H. contortus*. More recently, von-Son-de-Fernex et al. (2012) evaluated extracts of other tropical fodder legumes including *Arachis pintoi*, different cultivars of *Cratilia argentea* and *Gliricidia sepium* and showed significant *in vitro* AH effect against different stages of *H. contortus*, confirming the role of tannins with the addition of polyethylene glycol. Other research groups followed a similar approach, testing for example *Manihot esculenta* leaves or *Musa paradisiaca* leaves which also showed a good AH activity against *H. contortus* (Marie-Magdeleine et al., 2010, 2014). Evidence obtained so far from the few plants species screened, suggests that there could be a large number of tropical plants with potential AH activity against GIN. However, the vast quantity of potential candidates without an evaluation makes it difficult to decide which plants should be screened. Thus, taking into consideration the interest on nutraceutical materials, meaning that animals have to eat the plant materials to obtain the AH effect, the first candidates to screen should be those that are readily consumed by ruminants with browsing experience. However, this approach still represents a complex decision because ruminants may eat a large number of plant species in the tropical forest and those plants are consumed with different levels of preference (González-Pech et al., 2014; 2015). Thus, the next decision step could be related to the level of preference shown by the animals. This approach could also help to analyse possible self-medication behaviour in the ruminant hosts (Villalba et al., 2014).

An entirely different approach consist on evaluating the AH activity of plants extracts that previously showed activity against other microorganisms. The *in vitro* AH activity of *Phytolacca icosandra* extracts against eggs and larvae of *H. contortus* was reported by Hernández-Villegas et al. (2011) and similar AH activity was reported for different plant species of the *Anonacea* family (Castañeda-Ramírez et al., 2014).

The *in vitro* screening of tropical plants has suggested that the PSM composition of tropical plants is more complex than that of temperate plants. Recent *in vitro* studies with a range of tropical tannin rich legumes and agroindustrial by-products showed that the AH activity was not totally related to their CT content as the use of PVPP or PEG failed to block the totality of the AH effect shown by some of the plant extracts tested (Chan-Pérez et al., 2015; Vargas-Magaña et al., 2014a). In some cases the AH activity was significantly enhanced after the tannins were blocked either with PVPP or PEG. The latter suggests that some plant extracts tested contain certain polyphenols that perform differently to those already described in temperate tannin-rich plants. This phenomenon warrants further research. Also, the evidence of such *in vitro* studies suggests the existence of antagonisms between PSM within a single plant. If such antagonism exist, then of course it could be possible to expect possible antagonisms between PSM when animals consume two or more plants. This is especially important when considering the future on-farm application in ruminants browsing the tropical forest.

Only a hand-full of pen or field trials have been performed to measure the AH activity against GIN of tropical fodders (see reviews by Alonso-Díaz et al., 2010a and Torres-Acosta et al., 2012). The first evidence in the literature refers to a trial by Kabasa et al. (2000) reporting an increase in faecal egg counts in mixed natural infected goats when receiving regularly PEG in browsing conditions. Two studies focusing on leaves of *Acacia cyanophylla* performed with naturally infected sheep found significant effects on GIN egg faecal excretion (Akkari et al., 2008a, 2008b). The role of tannins on the reduction of GIN eggs was confirmed with the use of PEG. However, the AH effect of *Acacia* trees seem to vary between different species. For instance, *A. nilotica* fodder failed to offer any AH effect for goats infected with *H. contortus*, while *A. karoo* fodder reduced faecal egg excretion and worm burden (Kahiya et al., 2003; Marume et al., 2012). Thus, it would be important to identify the factors affecting the AH efficacy of different plants of the same species and also the variation between different species within the same genus.

The *in vivo* studies using the fodder of *L. latisiliquum* showed a reduction in the establishment of *H. contortus* and *T. colubriformis* L3 larvae in goats (Brunet et al., 2008). A further trial in sheep with established *H. contortus* populations reported a reduction of the faecal egg excretion as well as a reduction of fecundity and size of the female worms for animals consuming such plant material, (Martínez-Ortíz de Montellano et al., 2010). Similar *in vivo* studies performed with the foliage of *H. albicans,* a fodder tree with very high tannin content showed non-consistent effects on the *H. contortus* faecal egg excretion (Galicia-Aguilar et al., 2012; Mendez-Ortíz et al., 2012) although it also reduced both the fecundity and size of female *H. contortus* worms (Galicia-Aguilar et al., 2012).

Several early studies aiming at confirming the AH effects of tannin rich resources on different GIN stages in sheep (Athanasiadou et al., 2000, 2001) or goats (Paolini et al., 2003a, 2003b; 2005a) were performed by drenching animals with quebracho, which is a bark extract from a south American tree (*Schinopsis spp*). In a similar line, several more recent *in vivo* studies used bark extracts obtained from tropical plants (Murare et al., 2012, Xhomfulana et al., 2009). The use of commercial bark extracts from *A. mearnsi* is the most common example of PSM rich extracts against GINs, much in the same way as herbal medicines. The first trial by Cenci et al. (2007) showed that a weekly dose of 18 g/kg for 13 weeks reduced the faecal egg count of sheep with a mixed GIN infection and even reduced the worm burden. Meanwhile, Minho et al. (2007) used a dose level of 1.6 g of extract/kg BW for only two days in sheep and reported a significant reduction in FEC and worm burdens of *H. contortus* but without any effect on *T. colubriformis*. The next studies with the same material by Max et al. (2009, 2010) failed to find a clear AH effect on the GIN infection of goats and the effect was low in the case of sheep, even when using a larger dose of extract than that applied by Cenci et al. (2007). The latest report using *A. mearnsi* extract also failed to show any clear AH activity against a mixed GIN infection in goats (Costa-Júnior et al., 2014). This lack of consistency in results can be due to factors of variations exposed under section 3.5 and Figure 5. Differences in host species (sheep *vs*. goats) should also been considered. While the question of dose level remains to be explored for *A. mearnsi* commercial extracts, other studies evaluated different dose levels for bark extract of *Albizia anthelmintica* (Gradé et al., 2008) using naturally infected sheep. They reported that a dose of 0.8 g per animal resulted in significant effects on FEC. Another type of plant extract was also tested under *in vivo* conditions with naturally infected goats. Hernández-Villegas et al. (2012) tested a dose of 250 mg of *P. icosandra* ethanolic extract/kg BW dosed inside gel capsules. These authors showed a reduction of GIN eggs larger than 70% compared to non-treated controls. Similar to the studies with *A. mearnsi*, there was no previous information leading to determine the correct dose level and/or the duration of the treatment for goats. Therefore, the dose level was chosen based on previous reports for crude ethanolic extracts of different plants against natural GIN infections of sheep (Ademola et al., 2004, 2005, 2006, 2007a, 2007b).

To summarize the information from tropical plants, the majority have shown some evidence of AH effect. However, in most cases the AH effect is not as strong as it is with the tannin containing temperate legumes. The lack of a clear AH effect from feeding bioactive tropical forages could be explained by different reasons**: i)** the structural features of tannins present in these plants might differ from those in sericea lespedeza and/or sainfoin (see section 3.1.5); **ii)** parasites of ruminants species in tropical regions might have adapted to the bioactive PSMs that are constantly present in the animal´s diet, becoming less susceptible (Calderón-Quintal et al., 2010); **iii)** consistent with some recent *in vitro* trials (Chan-Pérez et al., 2015; Vargas-Magaña et al., 2014b) plants possess different PSMs, some of which might have antagonic effects that limit the potential AH effect of other PSMs. Interactions between PSMs have been previously described (Burrit and Provenza, 2000; Lyman et al., 2008) but are usually overlooked. However, under the conditions of the tropical forest, interactions between PSM are highly probable since browsing animals ingest a "cocktail of PSMs". This complex question needs further investigations.

Finally, the work with tannin rich foliage suggests that animals have the ability to eat large quantities of foliage for several days without any detrimental consequences on animal health but there are studies showing a trade-off that imply a negative effect on the diet digestibility due the consumption of tannin-rich foliage (Galicia-Aguilar et al., 2012; Mendez-Ortíz et al., 2012). The latter is consistent with the antinutritional nature of some PSM such as CT and saponins at high concentrations. In spite of such antinutritional evidence, the same studies suggested that sheep artificially infected with *H. contortus* might eat significantly more tannin rich foliage than non-infected animals (Martínez-Ortiz-de-Montellano et al., 2010; Mendez-Ortíz et al., 2012). The latter is consistent with potential self-medication behaviour (see reviews by Hutching et al., 2003; Villalba and Provenza, 2007; Junhke et al., 2012; Villalba et al., 2014).

3.7.3 Exploring the value of agro-industrial by-products

Variability in results (see Sub-section 3.6) is one of the key issues favouring the implementation of tannin-containing nutraceuticals in the integrated control of GINs worldwide. To pursue basic research on the mode of actions of tannins and flavonoids against digestive nematodes is a first step toward addressing this issue. A second important area of research is the continued exploration of different technological processes aimed at conserving and stabilising nutraceutical resources, in particular their PSM contents (see Sub-section 3.1.7). In regards to the use of nutraceuticals under farm conditions and in veterinary sciences, the questions of sustainability and cost of production have also to be considered.

The definition of plant nutraceuticals in veterinary sciences implies that the effect depends on the feed intake by animals. The main hypothesis is that the health effects relate to the presence of PSMs (Hoste et al., 2015). It is usually assumed that the active PSMs are intrinsic to the botanical resources exploited as nutraceuticals, such as with tannin-containing legumes. However, a second option is to add some resources ("part of the feed") with high concentrations of active PSMs. This option explains that, worldwide, the interest has been growing in exploring the potential of tannin-containing "by-products" from agro-industries. Until now, these by-products have generally been considered as "waste". However, they represent a potential "goldmine" of PSMs that might be able to be added to animal feed.

Regarding the worldwide potential applications for on-farm use, this approach, which aims at exploiting and/or including 'waste' tannin-rich materials into nutraceutical feeds for antiparasitic properties, has several potential advantages: (**1**) by solving the problem of the inherent variability of PSM contents in nutraceutical plants. The use of stored by products would allow measuring active PSMs before use, hence allowing the bioactive PSM concentration(s) in feeds to be adjusted (Girard et al., 2013), to achieve positive effects (including antiparasitic activity) without any side-effects caused by an excess of PSMs; **2**) by securing the production of low cost, tannin containing resources; **3**) by adding extra value to agro-industrial products, which until now, have generally been considered as "waste".

Therefore, many tannin-containing by-products from different areas of the world have been under investigation using *in vitro* assays, in order to screen the AH activities. Some examples are mentioned, to illustrate the potential exploitation of such by-products and the diversity of industrial by products in different areas of the world: (**1)** by-products from the nuts (hazelnut or chestnut) industry in temperate areas (Desrues et al., 2012, Girard et al, 2013); (**2)** carob pods and pistacia (Arroyo-Lopez et al., 2014; Manolaraki et al., 2010) around the Mediterranean Sea; and **(3)** by-products from tropical resources, including coffee and cocoa fruit husks and leaves (Covarrubias-Cárdenas et al., 2013; Vargas-Magaña et al., 2014a), banana leaves (*Musa x paradisiaca)* (Marie-Magdeleine, 2010), cassava leaves (*Manihot esculenta*) (Marie-Magdeleine et al., 2014; Seng et al., 2001, 2003, Sokerya et al., 2009) or of shea nuts' meal (*Vitellaria paradoxa*) (Ramsay et al., 2015) from tropical resources.

3.7.4 Possible combinations of resources with anthelmintic effects

- Combining different CT-containing resources?

The rationale for this option relates to the results presented in Sub-section 3.6. The idea is to improve the AH effects agaisnt GINs, either by increasing the overall diet content of CT and/or by combining resources with different tannins types (cf. Table 5) (e.g. with different proldephinidins and/or procyanidins ratios or the mean degree of polymerisation.

A study combining the use of sainfoin silage and faba beans (Werne et al., 2013) was amongst the first to explore such combinations (cf. Section 3.1.7). Another study, examining the possibility of associating sainfoin pellets (mainly prodelphionindins) and by-products of hazel nuts (mainly procyanindins), was conducted in lambs experimentally infected with *H. contortus* (see Girard et al., 2013). The results suggested that the addition of hazel nut peel to the sainfoin diet led to a reduction in FECs, towards significant values.

The use of mixtures of *L. cuneata* (prodelphinidins) plus pine bark powder (procyanidins) and also of *Ficus infectoria* plus *Psidum guajava* leaves resulted in lower worm numbers (Pathak et al., 2013; Wright, 2015). However, a mixture of *P. lentiscus*, *Arbutus unedo* and *Quercus ilex* proved ineffective (Saric et al., 2015), which might be linked to the fact that the adult sheep -rather than lambs - were fed and that they had acquired mixed natural infections of low intensity. The present concept is attractive, and seems worthy of further exploration under different epidemiological conditions. However, presently, there is a paucity of information, and no clear trend has been observed. This is not surprising since the idea relies mainly on potential interactions between different bioactive compounds; the complexity of such issues has been explained previously.

- Combining CT-containing nutraceuticals and synthetic chemical anthelmintics?

The question as to the possible interactions between nutraceuticals and the synthetic chemical AHs is of considerable practical importance, but has received little attention until recently. A first *in vitro* study, measuring the motility of larvae, suggested that extracts of redberry juniper (*Juniperus pinchotii*) could increase the activity of ivermectin against *H. contortus* (Armstrong et al., 2013). These first results were then confirmed in an *in vivo* study, showing that feeding lambs a diet containing 30% redberry juniper reduced FECs and increased ivermectin efficacy by 65% (Whitney et al., 2013). With similar objectives, another study explored the effects of the consumption of sainfoin pellets on a multiresistant strain of *H. contortus.* In contrast to previous data, the FEC results suggested a lower efficacy of oral ivermectin in lambs consuming sainfoin. These results were confirmed by changes in pharmacokinetic parameters of the drug in the blood (Gaudin et al., 2015). It is worth noting that, besides CT and flavonoids, terpenoids are also present in redberry juniper, which is not the case for sainfoin. Clearly, further studies are needed to explore these interactions and to better understand the specific mechanisms associated with these interactions between the polyphenolic compounds and the pharmacology of macrocyclic lactones (Dupuy et al., 2003).

4. CONCLUSIONS

The almost exclusive reliance on treating animals with synthetic anthelminitic drugs is seriously challenged nowadays, because of the high adaptive capacity of nematodes, illustrated by the constant and rapid development of resistance in worm populations to these drugs. These facts indicate that a more sustainable mode of GIN control is now required, which should not be based on just one option/principle of control (Hoste and Torres-Acosta, 2011). Instead, an integrated control of nematodes is currently recommended, which is based on a combination of different components. The manipulation of host nutrition is one of the key components. However, a better understanding of grazing management and also breeds or lines with genetic resistance, new synthetic drugs, new modes of application of current synthetic drugs and, hopefully, vaccines will also be needed and are expected. These different items relate to the different chapters of this Special Issue of *Advances in Parasitology*.

In the current chapter, we illustrated the complexity of interactions between host nutrition and *H. contortus* and related nematodes in small ruminants. We also highlighted that a better understanding of these interactions is likely to offer a range of solutions by exploiting, across the world, the contributions that host nutrition can make. *"If you take care of the nutrition of your livestock animals, then they will take care of the parasite threat".* This sentence stems from traditional experience and common sense. It emphasises the possible benefits that better nutrition could achieve in animal production and health - and this has been recognised for a long time. In the case of *H. contortus* infection in lambs and kids, improved nutrition can prevent animal deaths, which is essential for small farmers, because, for them, this is synonymous with animal production.

The first scientific studies that validated quantitative aspects of manipulating host nutrition were initiated by Clunies-Ross (1932). Qualitative aspects, namely nutraceutical PSMs commenced some 20 years ago. Finally, it is expected that related studies of self-medication will probably become more important in the future.

To address the basic questions regarding the manipulation of nutrition for GIN control requires not only parasitological studies, but also multidisciplinary investigations, which will, for example, link research to nutrition, immunology, phytochemistry and/or pharmacology. Across the world, basic studies of the exact mechanisms of action will help identify suitable solutions for a range of epidemiological and agronomic problems. This focus will also assist extending to studies of other animal species, such as cattle, camelids and exotic ruminants.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the financial support of the European Commission through the "LegumePlus" project (PITN-GA-2011-289377), the EMIDA ERANET project CARES, the INRA métaprogramme GISA STREP, the CORE ORGANIC 2 ProPARA and the PCP France-Mexico project 2013-2017 (Fondo Institucional CONACYT No. 229330). Also, the authors wish to express their sincere thanks to Pr. R. B. Gasser for his constant and efficient help to edit this chapter

REFERENCES

Abbott, E.M., Parkins, J.J., Holmes, P.H., 1985a. Influence of dietary protein on parasite establishment and pathogenesis in Finn Dorset and Scottish Blackface lambs given a single moderate infection of *Haemonchus contorus*. Res. Vet. Sci. 38, 6-13.

Abbott, E.M., Parkins, J.J., Holmes, P.H., 1985b. Influence of dietary protein on the pathophysiology of ovine haemonchosis in Finn Dorset and Scottish Blackface lambs given a single moderate infection. Res. Vet. Sci. 38, 54-60.

Abbott, E.M., Parkins, J.J., Holmes, P.H., 1986a. The effect of dietary protein on the pathogenesis of acute ovine haemonchosis. Vet. Parasitol. 20, 275-289.

Abbott, E.M., Parkins, J.J., Holmes, P.H., 1986b. The effect of dietary protein on the pathophysiology of acute ovine haemonchosis. Vet. Parasitol. 20, 29l-306.

Abbott, E.M., Parkins, J.J., Holmes, P.H., 1988. Influence of dietary protein on the pathophysiology of haemonchosis in lambs given continuous infections. Res. Vet. Sci. 45, 41–49.

Abbott, E.M., Holmes, P.H., 1990. Influence of dietary protein on the immune responsiveness of sheep to *Haemonchus contortus.* Res. Vet. Sci. 48, 103-107.

Acharya, J., Hildreth, M.B., Reese, R.N., 2014. *In vitro* screening of forty medicinal plant extracts from the United States Northern Great Plains for anthelmintic activity against *Haemonchus contortus*. Vet. Parasitol. 201, 75-78.

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2004. Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 122, 151–164.

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2005. Anthelmintic activity of extracts of *Spondias mombin* against gastrointestinal nematodes of sheep: studies *in vitro* and *in vivo*. Trop. Anim. Health Prod. 37, 223–235.

Ademola, I.O., Idowu, S.O., 2006. Anthelmintic activity of *Leucaena leucocephala* seed extract on *Haemonchus contortus* infective larvae. Vet. Rec. 158, 485–486.

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2007a. Anthelmintic activity of *Spigelia anthelmia* extract against gastrointestinal nematodes of sheep. Parasitol. Res. 101, 63–69.

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2007b. Anthelmintic efficacy of *Nauclea latifolia* extract against gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Afr. J. Trad. 4, 148–156.

AFRC (Agricultural and Food Research Council), 1993. Energy and protein requirements of ruminant livestock. CAB International, Wallingford, UK.

Aguilar-Caballero, C.A.J., Torres-Acosta, J.F., Vera-Ayala, C., España-España, E., 2002. Long and short-term supplementary feeding and the resilience of browsing Criollo goats to gastrointestinal nematodes. In: Responding to the increasing global demand for animal products. Brit. Soc. Anim. Sci. 12–15 November 2002. Mérida, México. pp. 213–214.

Akkari, H., Ben Salem, H., Gharbi, M., Abidi, S., Darghouth, M.A., 2008a. Feeding *Acacia cyanophylla* Lindl. foliage to Barbarine lambs with or without PEG: effect on the excretion of gastro-intestinal nematode eggs. Anim. Feed Sci. Technol. 147, 182–192.

Akkari, H., Darghouth, M.A., Ben Salem, H., 2008b. Preliminary investigations of the anti-nematode activity of *Acacia cyanophylla* Lindl.: excretion of gastrointestinal nematode eggs in lambs browsing *A. cyanophylla* with and without PEG or grazing native grass. Small Ruminant Res. 74, 78–83.

Albers, G. A. A., Gray, G. D., Piper, L.R., Barker, J.S.F., Le Jambre, L.F., Barger, I. A., 1987. The genetics of resistance and resilience to *Haemonchus contortus* infection in young Merino sheep. Int. J. Parasitol. 17, 1355-1363.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar Caballero, A.J., Hoste, H., 2008a. *In vitro* larval migration and kinetics of exsheathment of *Haemonchus contortus* exposed to four tropical tanniniferous plant extracts. Vet. Parasitol. 153, 313–319.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Capetillo-Leal, C.M., Brunet, S., Hoste, H., 2008b. Effects of four tropical tanniniferous plant extracts on the inhibition of larval migration and the exsheathment process of *Trichostrongylus colubriformis* infective stage. Vet. Parasitol. 153, 187-192.

Alonso-Díaz M.A., Torres-Acosta J.F.J., Sandoval-Castro C.A., Capetillo-Leal, C.M., 2010b. Polyphenolic compounds of nutraceutical trees and the variability of their biological activity measured by two methods. Trop. Subtrop. Agroecosyst. 12, 649-656.

Alonso-Diaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010a. Tannins in tanniniferous tree fodders fed to small ruminants: a friendly foe? Small Ruminant Res. 89, 164–173.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2011. Comparing the sensitivity of two *in vitro* assays to evaluate the anthelmintic activity of tropical tannin rich plant extracts against *Haemonchus contortus.* Vet. Parasitol. 181, 360-364.

Al-Qaizy, H.H.K., Altaif, K.I., Al-Zubaidy, A. J., Makkawi, T.A., 1986. Pathogenicity of haemonchosis due to *H. contortus* in sheep and goats in Iraq*.* Vet. Parasitol. 21, 249-256.

Al-Zubaidy, A.J., Altaif, K.I., Al-Qaizy, H.H.K., Makkawi, T.A., 1987. Gross pathology and histopathology of haemonchosis in sheep and goats in Iraq. Vet. Parasitol. 23, 249-256.

Amarante, A.F.T., 2014. Sustainable worm control practices in South America. Small Ruminant Res. 118, 56-62.

Andlauer, W., Fürst, P., 2002. Nutraceuticals: a piece of history, present status and outlook. Food Res. Int. 35, 171–176.

Armstrong, S.A., Klein, D.R., Whitney, T.R., Scott, C.B., Muir, J.P., Lambert, B.D., Craig, T.M., 2013. Effect of using redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus* in vitro motility and increase ivermectin efficacy. Vet. Parasitol. 197, 271-276.

Arroyo-Lopez, C., Manolaraki, F., Saratsis, A., Saratsi, K., Stefanakis, A., Skampardonis, V., Voutzourakis, N., Hoste, H., Sotiraki, S., 2014. Anthelmintic effect of carob pods and sainfoin hay when fed to lambs after experimental trickle infections with *Haemonchus contortus* and *Trichostrongylus colubriformis*. Parasite, 21, 71-80.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2000. Consequences of long-term feeding with condensed tannins on sheep parasitized with *Trichostrongylus colubriformis.* Int. J. Parasitol. 30, 1025−1033.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. Vet. Parasitol. 99, 205−219.

Athanasiadou, S., Gray, D., Younie, D., Tzamaloukas, O., Jackson, F., Kyriazakis, I., 2007. The use of chicory for parasite control in organic ewes and their lambs. Parasitology, 134, 299-307.

Azaizeh, H., Halahleh, F., Abbas, N., Markovics, A., Muklada, H., Ungar, E.D., Landau, S.Y., 2013. Polyphenols from *Pistacia lentiscus* and *Phillyrea latifolia* impair the exsheathment of gastro-intestinal nematode larvae. Vet. Parasitol. 191, 44-50.

Azando, E.V.B., Hounzangbé−Adoté, M.S., Olounladé, P.A., Brunet, S., Fabre, N., Valentin, A., Hoste, H., 2011. Involvement of tannins and flavonoids in the *in vitro* effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloïdes* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. Vet. Parasitol. 180, 292−297.

Azuhnwi, B. N., 2012. Cultivar differences in nutritional and anthelmintic potential of sainfoin (*Onobrychis viciifolia*) in ruminants. Phd thesis ETH Zurich, 130 pages.

Azuhnwi, B.N., Boller, B., Dohme-Meier, F., Hess, H.D., Kreuzer, M., Stringano, E., Mueller-Harvey, I., 2013a. Exploring variation in proanthocyanidin composition and content of sainfoin (*Onobrychis viciifolia*). J. Sci. Food Agric*.* 93, 2102-2109.

Azuhnwi, B. N., Hertzberg, H., Arrigo, Y., Gutzwiller, A., Hess, H. D., I. Mueller-Harvey, I., Torgerson, P.R., Kreuzer, M., Dohme-Meier, F., 2013b. Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs. J. An. Sci. 91, 2343-2354.

Bachaya, H.A., Iqbal, Z., Khan, M.N., Sindhu, Z., Jabbar, A., 2009. Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against trichostrongylid nematodes of sheep. J. Ethnopharmacol. 123, 325−329.

Bahuaud, D., Martinez-Ortiz-de-Montellano, C., Chauveau, S., Prevot, F., Torres-Acosta, J.F.J., Fouraste, I., Hoste, H., 2006. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. Parasitology 132, 545−554.

Balic, A., Bowles, V.M., Meeusen, E.N., 2000. The immunobiology of gastrointestinal nematode infections in ruminants. Adv. Parasitol. 45, 181-241.

Bambou, J.C., Arquet, R., Archimède, H., Alexandre, G., Mandonnet, N., Gonzalez-Garcia, E., 2009. Intake and digestibility of naive kids differing in genetic resistance and experimental parasitized (indoors) with *Haemonchus contortus* in two successive challenges. J. Anim. Sci. 87, 2367-2375.

Bambou, J.C., Archimède, H., Arquet, R., Mahieu, M., Alexandre, G., Gonzalez-Garcia, E., Mandonnet, N., 2011. Effect of dietary supplementation on resistance to experimental infection with *Haemonchus contortus* in Creole kids. Vet. Parasitol. 178, 279-285.

Bambou, J.C., Cei, W., Camous, S., Archimède, H., Decherf, A., Philibert, L., Barbier, C., Mandonnet, N., Gonzalez-Garcia, E., 2013. Effects of single or trickle *Haemonchus contortus* experimental infection on digestibility and host responses of naïve Creole kids reared indoor. Vet Parasitol. 191, 284-292.

Bang, K.S., Familton, A.S., Sykes, A.R., 1990. Effect of copper oxide wire particle treatment on establishment of major gastro intestinal nematodes in lambs. Res. Vet. Sci. 49, 132-137.

Barbehenn, R.V., Jones, C.P, Hagerman, A.E., Karonen, M., Salminen, J.-P., 2006. Ellagitannins have greater oxidative activities than condensed tannins and galloyl glucoses at high pH: potential impact on caterpillars. J. Chem. Ecol. 32, 2253−2267.

Barger, I. A., 1993. Influence of sex and reproductive status on susceptibility of ruminants to nematode parasitism. Int. J. Parasitol. 23, 463-469.

Barrau, E., Fabre, N., Fouraste, I., Hoste, H., 2005. Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia* scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitology 131, 531-538.

Betts, M.J., Brown, B.R., Brown, P.E., Pike, W.T., 1967. Degradation of condensed tannins: structure of the tannin from common heather. J. Chem. Soc. Chem. Comm. 1110-1112.

Blackburn, H.D., Rocha, J.L., Figueiredo, E.P., Berne, M.E., Vieira, L.S., Cavalcante, A.R., Rosa, J.S., 1991. Interaction of parasitism and nutrition and their effects on production and clinical parameters in goats. Vet. Parasitol. 40, 99−112.

Blackburn, H.D., Rocha, J.L., Figueiredo, E.P., Berne, M.E., Vieira, L.S., Cavalcante, A.R., Rosa, J.S., 1992. Interaction of parasitism and nutrition in goats: effects on haematological parameters, correlations, and other statistical associations. Vet. Parasitol. 44, 183−197.

Bouquet, W., Hoste, H., Chartier, C., Coutineau, H., Koch, C., Pors, I., 1997. Effects of dietary supplementation with methionine in dairy goats infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. The British Society for Parasitology, University of Manchester, Institute of Science and Technology, p. 77.

Bown, M.D., Poppi, D.P., Sykes, A.R., 1991. The effect of post ruminal infusion of protein or energy on the pathophysiology of *Trichostrongylus colubriformis* infection and body composition in lambs. Aust. J. Agric. 42, 253–267.

Bricarello, P.A., Amarante, A.F.T., Rocha, R.A., Cabral-Filho, S.L., Huntley, J.F., Houdijk, J.G.M., Abdalla, A.L., Gennari, S.M., 2005. Influence of dietary protein supply on resistance to experimental infections with *Haemonchus contortus* in Ile de France and Santa Ines lambs. Vet. Parasitol. 134, 99–109.

Brunet, S., Hoste, H., 2006. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. J. Agri. Food Chem. 54, 7481-7487.

Brunet, S., Aufrère, J., El Babili, F., Fouraste, I., 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., Jackson, F., Hoste, H., 2008a. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. Int. J. Parasitol. 38, 783–790.

Brunet, S., Martinez-Ortiz De Montellano, C., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar – Caballero, A.J., Capetillo-Leal, C.M., Hoste, H., 2008b. Effect of the consumption of *Lysiloma latisilliquum* on the larval establishment of parasitic nematodes in goats. Vet. Parasitol. 157, 81-88.

Brunet, S., Fourquaux, I., Hoste, H., 2011. Ultrastructural changes in the infective third-stage larvae of parasitic nematodes of ruminants treated with a sainfoin (*Onobrychis viciifolia*) extract. Parasitol. Int. 60, 419-424.

Bundy, D.A., Golden, M.H., 1987. The impact of host nutrition on gastrointestinal helminth populations. Parasitology, 95, 623-635.

Burke, J.M., Miller, J.E., Mosjidis, J.A., Terrill, T.H., 2012a. Grazing sericea lespedeza for control of gastrointestinal nematodes in lambs. Vet. Parasitol. 186, 507-512*.*

Burke, J.M., Miller, J.E., Mosjidis, J.A., and Terrill, T.H., 2012b. Use of a mixed sericea lespedeza and grass pasture system for control of gastrointestinal nematodes in lambs and kids. Vet. Parasitol. 186, 328-336*.*

Burrit, E.A., Provenza, F.D., 2000. Role of toxins in intake of varied diets by sheep. J. Chem. Ecol. 26, 1991–2005

Calderón-Quintal, J.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Alonso- Díaz, M.A., Hoste, H., Aguilar-Caballero, A., 2010. Adaptation of *Haemonchus contortus* to condensed tannins: can it be possible? Arch. Med. Vet. 42, 165–171.

Castañeda-Ramírez, G.S., Torres-Acosta, J.F.J., Mendoza-de-Gives, P., Chan-Pérez, J.I., Tun-Garrido, J., Rosado-Aguilar, J.A., 2014. *In vitro* anthelmintic effect of the foliage from three plant species of the Annonaceae family against *Haemonchus contortus*. In: 13th International Congress of Parasitology, August 10–15, 2014. México City, México.

Cenci, F.B, Louvandini, H., McManus, C.M., Dell'Porto, A., Costa, D.M., Arau´jo, S.C., Minho, A.P., Abdalla, A.L., 2007. Effects of condensed tannin from *Acacia mearnsii* on sheep infected naturally with gastrointestinal helminthes. Vet Parasitol. 144, 132-137.

Chan-Pérez, J.I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A. Hoste, H., Castañeda-Ramírez, G.S., Vilarem, G., Mathieu, C., 2015. Phenotypic variability between *Haemonchus contortus* isolates from different geographical origins. Effect of polyphenols from acetone:water extracts on egg development. Vet. Parasitol. Submitted.

Chartier, C., Hoste, H., 1994. Anthelmintic treatments against digestive-tract nematodes in grazing dairy goats with high or low levels of milk production. Vet. Res. 25, 450-457.

Chartier, C., Hoste, H., 1997. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats: differences between high-and low-producers. Vet. Parasitol. 73, 267-276.

Chartier, C., Hoste, H., Bouquet, W., Malpaux, B., Pors, I., Koch, C., 1998. Periparturient rise in fecal egg counts associated with prolactin concentration increase in French Alpine dairy goats. Parasitol. Res. 84, 806-810.

Chartier, C., Etter, E., Hoste, H., Pors, I., Mallereau, M.P., Broqua, C., Mallet, S., Koch, C., Masse, A., 2000. Effects of the initial level of milk production and of the dietary protein intake on the course of natural nematode infection in dairy goats. Vet. Parasitol. 92, 1–13.

Chartier, C., Etter, E., Hoste, H., Pors, I., Koch, C., Dellac, B., 2000. Efficacy of copper oxide needles for the control of nematode parasites of dairy goats. Vet. Res. Comm. 24, 389-399.

Clark, C.H., Kiesel, G.K., Goby, C.H., 1962. Measurements of blood loss caused by *Haemonchus contortus* infection in sheep. Am. J. Vet. Res. 23, 977-980.

Clunies-Ross, I., 1932. Observations on the resistance of sheep to infestation by the stomach worm (*Haemonchus contortus*). CSIR J., 5, 74-80.

Combes, C., 1995. Interactions durables. Ecologie et évolution du parasitisme. Masson ed., Paris. 524 p.

Coop, R.L., Field, A.C., 1983. Effect of phosphorus intake on growth rate, food intake and quality of skeleton of growing lambs infected with the intestinal nematode: *Trichostrongylus vitrinus*. Res. Vet. Sci., 35, 175-181.

Coop, R.L., Huntley, J.F., Smith, W.D., 1995. Effect of dietary protein supplementation on the development of immunity to *Ostertagia circumcincta* in growing lambs. Res. Vet. Sci. 59, 24–29.

Coop, R.L., Holmes, P.H., 1996. Nutrition and parasite interaction. Int. J. Parasitol. 26, 951–962.

Coop, R.L., Kyriazakis, I., Huntley, J.F., Jackson, E., Jackson, F., 1997. The influence of protein and amino acid on the resilience of sheep to intestinal parasitism. In: Proc. 4th International Congress for Sheep Veterinarians, February 1997, Armidale, N.S.W., Australia, pp. 196–198.

Coop, R.L., Kyriazakis, I., 1999. Nutrition-parasite interaction. Vet. Parasitol. 84, 187–204.

Coop, R.L., Kyriazakis, I.K., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. Trends Parasitol. 17, 325-330.

Copani, G., Ginane, C. Le Morvan, A., Niderkorn, V. 2014. Patterns of *in vitro* rumen fermentation of silage mixtures including sainfoin and red clover as bioactive legumes. An. Feed Sci. Techn., 208, 220-224.

Costa, C.A.F., 1983. Increase of gastro intestinal nematode egg counts in lactating goats. Pesq. Agrospec. Bras., 18, 919-929.

Covarrubias-Cárdenas, A.G., Torres-Acosta, J.F.J, Sandoval-Castro, C.A., Hoste, H., 2013. *In vitro* anthelmintic effect of *Acacia pennatula* and *Coffea arabica* extracts on *Haemonchus contortus* sensitive to tannins. 7th Novel Approaches to the Control of Helminths of Livestock. 25-28th March. Toulouse, France.

Costa, C.T.C., Morais, S.M., Bevilaqua, C.M.L., Souza, M.M.C., Leite, F.K.A., 2002. Efeito ovicida de extratos de sementes de *Mangifera indica* l. sobre *Haemonchus contortus*. Rev. Bras. Parasitol. Vet. 11, 57–60.

Costa-Júnior, L.M., Costa, J.S., Lôbo, T.C.P.D., Soares, A.M.S., Abdala, A.L., Chaves, D.P., Batista, Z.S., Louvandini, H. 2014. Long-term effects of drenches with condensed tannins from *Acacia mearnsii* on goats naturally infected with gastrointestinal nematodes. Vet. Parasitol. 205, 725-729

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K., 2014. *Pistacia lentiscus* leaves as a source of phenolic compounds: microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Ind. Crops Prod. 61, 31-40.

Dakkak, A., 1984. Physiopathologie digestive des trichostrongylidoses ovines = physiopathologie digestive de l'haemonchose ovine: revue bibliographique. Rev. Med. Vet. 135, 459-467.

Dargie, J.D., Allonby, E.W., 1975. Pathophysiology of single and challenge infections of *Haemonchus contortus* in Merino sheep: studies on red cell kinetics and the "self cure" phenomenon. Int. J. Parasitol. 5, 147-157.

Datta, F.U., Nolan, J.V., Rowe, J.B., Gray, G.D., 1998. Protein supplementation improves the performance of parasitised sheep fed a straw-based diet. Int. J. Parasitol. 28, 1269–1278.

Datta, F.U., Nolan, J.V., Rowe, J.B., Gray, G.D., Crook, B.J., 1999. Long-term effects of short-term provision of protein-enriched diets on resistance to nematode infection, live weight gain and wool growth in sheep. Int. J. Parasitol. 29, 479–488.

Debela, E., Tolera, A., Eik, L.O., Salte, R., 2012. Condensed tannins from *Sesbania sesban* and *Desodium intortum* as a means of *Haemonchus contortus* control in goats. Trop. Anim. Health Prod. 44, 1939-1944.

Del Rio, D., Calani, L., Dall Asta, M., Brighenti, F., 2011. Polyphenolic composition of hazelnut skin. J. Agric. Food Chem. 59, 9935-9941.

Desrues, O., Vargas-Magaña, J.J., Girard, M., Manolaraki, F., Pardo, E., Mathieu, C., Vilarem, G., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Jean, H., Hoste H., 2012. Can hazel-nut peels be used to control gastrointestinal nematodes in goats? 11th International Goat Conference. Las Canarias, Sept 2012.

Desrues, O., Peña-Espinoza, M., Hansen, T.V., Mueller-Harvey, I., Enemark, H.L., Thamsborg, S.M., 2015. Anthelmintic effects of sainfoin against different cattle nematodes may be linked to concentration of condensed tannins in different gut compartments. 25th WAAVP conference. Liverpool, August 2015.

Desrues, O., Fryganas, C., Ropiak, H.M., Mueller-Harvey, I., Enemark, H.L., Thamsborg, S.M. 2016. Impact of chemical structure of flavanol monomers and condensed tannins on *in vitro* anthelmintic activity against bovine nematodes. Parasitology, In press.

Diaz Lira, C.M., Barry, T.N., Pomroy, W.E., McWilliam, E.L., Lopez-Villalobos, N., 2008. Willow (*Salix* spp.) fodder blocks for growth and sustainable management of internal parasites in grazing lambs. Anim. Feed Sci. Technol. 141, 61−81.

Donaldson, J., Van-Houtert, H.F.J., Sykes, A.R., 1998. The effect of nutrition on the periparturient parasite status of mature ewes. Anim. Sci. 67, 523−533.

Dobreva, M.A., Frazier, R.A., Mueller-Harvey, I., Clifton, L.A., Gea, A., Green, R.J., 2011. Binding of pentagalloyl glucose to two globular proteins occurs via multiple surface sites. Biomacromolecules, 12, 710-715.

Dube, J.S., Ndlovu, L.R., 1995. Feed intake, chemical composition of faeces and nitrogen retention in goats consuming single browse species or browse mixtures. Zimbabwe J. Agric. Res. 33, 133−141.

Dube, J.S., Reed, J.D., Ndlovu, L.R., 2001. Proanthocyanidins and other phenolics in *Acacia* leaves of Southern Africa. Anim. Feed Sci. Technol. 91, 59−67.

Dupuy, J., Larrieu, G., Sutra, J.F., Lespine, A., Alvinerie, M., 2003. Enhancement of moxidectin bioavailability in lamb by a natural flavonoid: quercetin. Vet. Parasitol. 112, 337-347.

Engström, M.T., Pälijärvi, M., Fryganas, C., Grabber, J.H., Mueller-Harvey, I., Salminen, J.-P., 2014. Rapid qualitative and quantitative analysis of proanthocyanidin oligomers and polymers by UPLC-MS/MS. J. Agric. Food Chem. 62, 3390-3399.

Etter, E., Chartier, C., Hoste, H., Pors, I., Bouquet, W., Lefrileux, Y., Borgida, L. P., 1999. The influence of nutrition on the periparturient rise in faecal egg counts in dairy goats: results from a 2-year study. Rev. Med. Vet. 150, 975−980.

Etter, E., Hoste, H., Chartier, C., Pors, I., Koch, C., Broqua, C., Coutineau, H., 2000. The effect of two levels of dietary protein on resistance and resilience of dairy goats experimentally infected with *Trichostrongylus colubriformis*: comparison between high and low producers. Vet. Res. 31, 247−258.

Fakae, B., Campbell, A.M., Barrett, J., Scott, I.M., Teesdale-Spittle, P.H., Liebau, E., Brophy, P.M., 2000. Inhibition of glutathione S-transferases (GSTs) from parasitic nematodes by extracts from traditional Nigerian medicinal plants. Phytother. Res. 14, 630−634.

Falchero, L., Brown, R.H., Karp, A., Hanley, S., Shield, I., Mueller-Harvey, I., 2011. The structural diversity of condensed tannins in willows (*Salix* spp.): a first screening to improve the nutritional quality of ruminant products. Adv. Anim. Biosci. 2, 410.

Faye, D., Leak, S., Nouala, S., Fall, A., Losson, B., Geerts, S., 2003. Effects of gastrointestinal helminth infections and plane of nutrition on the health and productivity of F1 (West African Dwarf x Sahelian) goats crosses in the Gambia. Small Ruminant Res. 50, 153−161.

Foo, L.Y., Jones, W.T., Porter, L.J., Williams, V.M., 1982. Proanthocyanidin polymers of fodder legumes. Phytochemistry 21, 933-935.

Foo, L.Y., Lu, Y., McNabb, W.C., Waghorn, G., Ulyatt, M.J., 1997. Proanthocyanidins from *Lotus peduculatus*. Phytochemistry, 45, 1689-1696.

Foo, L.Y., Newman, R., Waghorn, G., McNabb, W.C., Ulyatt, M.J. 1996. Proanthocyanidins from *Lotus corniculatus*. Phytochemistry 41, 617−624.

Foster, J.G., Cassida, K.A., Sanderson, M.A., 2011a. Seasonal variations in sesquiterpene lactone concentrations and composition of forage chicory (*Cichorium intybus L.*) cultivars. Grass Forage Sci. 66, 424-433.

Foster, J.G., Joyce, G., Cassida, K.A., Turner, K.E., 2011b. *In vitro* analysis of the anthelmintic activity of forage chicory (*Cichorium intybus L.*) sesquiterpene lactones against a predominantly *Haemonchus contortus* egg populations. Vet. Parasitol. 180, 296-306.

Fox, M.T. 1993. Pathophysiology of infection with *Ostertagia ostertagi* in cattle. Vet. Parasitol. 46, 143-158.

Freer, M., Moore, A.D., Donnelly, J.R., 1997. GRAZPLAN: decision support systems for Australian grazing enterprises. II. The animal biology model for feed intake, production and reproduction and the GrazFeed DSS. Agric. Syst. 54, 77−126.

Galicia-Aguilar, H.H., Rodríguez-González, L.A., Capetillo-Leal, C.M., Cámara-Sarmiento, R., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Torres-Acosta, J.F.J., 2012. Effect of *Havardia albicans* supplementation on feed consumption and dry matter digestibility of sheep and the biology of *Haemonchus contortus*. Anim. Feed Sci. Technol. 176, 178-184.

Gárate-Gallardo. L., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Cámara-Sarmiento, R., Canul-Ku, H.L., 2015. Comparing different maize supplementation strategies to improve resilience and resistance against gastrointestinal nematode infections in browsing goats. Parasite, 22, 19.

Gaudin, E., Simon, M., Quijada, J., Varady, M., Lespine, A., Schelcher, F., Hoste, H., 2015. Effect of sainfoin (*Onobrychis viciifolia*) pellets on a multi-resistant strain of *Haemonchus contortus* in lambs. 25th WAAVP Conference, Liverpool, UK, 16th-20th August 2015.

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey, I., 2011. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high throughput germplasm screening. J. Agric. Food Chem. 59, 495-503.

Gennari, S.M., Vieira Bressan, M.C.R., Rogero, J.R., Mac Lean, J.M., Duncan, J.L., 1991. Pathophysiology of *Haemonchus placei* infection in calves. Vet. Parasitol. 38, 163-172.

Girard, M., Gaid, S., Mathieu, C., Vilarem, G., Gerfault, V., Routier, M., Gombault, P., Pardo, E., Manolaraki, F., Hoste, H., 2013. Effects of different proportions of sainfoin pellets combined with hazel nut peels on infected lambs. 64th EAAP Nantes, 26th -30th August 2013, p. 506.

Gonzalo-Garduno, R., Torres-Acosta, J.F.J., Chay-Canul, A.J., 2014. Susceptibility of hair sheep ewes to nematode parasitism during pregnancy and lactation in a selective anthelmintic treatment scheme under tropical conditions. Res. Vet. Sci., 96, 487-492.

González-Pech, P.G., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Tun-Garrido, J., 2015. Feeding behavior of sheep and goats in a deciduous tropical forest during the dry season: the same menu consumed differently. Small Ruminant Res. Accepted.

González-Pech, P.G., Torres-Acosta J.F.J., Sandoval Castro, C.A., 2014. Adapting a bite coding grid for small ruminants browsing a deciduous tropical forest. Trop. Subtrop. Agroecosyst. 17, 63-70.

Grabber, J., Zeller, W.E., Mueller-Harvey, I., 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in *Lotus* species by the butanol-HCl-iron assay. J. Agric. Food Chem. 61, 2669-2678.

Grabber, J.H., Riday, H., Cassida, K.A., Griggs, T.C., Min, D.H., MacAdam, J.W., 2014. Yield, morphological characteristics, and chemical composition of European- and Mediterranean-derived birdsfoot trefoil cultivars grown in the colder continental United States. Crop Sci. 54, 1893−1901.

Gradé, J.T., Arble, B.L., Weladji, R.B., Van Damme, P., 2008. Anthelmintic efficacy and dose determination of *Albizia anthelmintica* against gastrointestinal nematodes in naturally infected Ugandan sheep. Vet. Parasitol. 157, 267−274.

Gujja, S., Terrill, T.H., Mosjidis, J.A, Miller, J.E., Mechineni, A., Kommuru, D.S., Shaik, S.A., Lambert B.D., Cherry, N.M., Burke, J.M., 2013. Effect of supplemental sericea lespedeza leaf meal pellets on gastro intestinal nematode infection in grazing goats. Vet. Parasitol. 191, 51-58.

Gutiérrez-Segura, I., Torres-Acosta, J.F., Aguilar-Caballero, A.J., Cob-Galera, L., May-Martínez, M., Sandoval-Castro, C., 2003. Supplementation can improve resilience and resistance of browsing criollo kids against nematode infections during the wet season. Trop. Subtrop. Agroecosyst. 3, 537−540.

Haile, A., Tembely, S., Anindo, D.O., Mukasa-Mugerwa, E., Rege, J.E.O., Yami, A., Baker, R.L., 2002. Effects of breed and dietary protein supplementation on the responses to gastrointestinal nematode infections in Ethiopian sheep. Small Ruminant Res. 44, 247−261.

Haile, A., Anindo, D.O., Tembely, S., Mukasa-Mugerwa, E., Tibbo, M., Yami, A., Baker, R.L., Rege, J.E.O., 2004. Effects of dietary protein supplementation and infection with gastrointestinal nematode parasites on some nutritional and metabolic parameters in Ethiopian Menz and Horro sheep. Livest. Prod. Sci. 91, 183−195.

Hayot Carbonero, C., Mueller-Harvey, I., Brown, T.A, Smith, L., 2011. Sainfoin (*Onobrychis viciifolia*): a beneficial forage legume. Plant Genet. Res.: Util. Charact. 9, 70-85.

Heckendorn, F., Häring, D.A.; Maurer, V., Zinsstag, J., Langhans W., Hertzberg, H., 2006. Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* and *Cooperia curticei* in lambs. Vet. Parasitol**,** 142, 293-300.

Heckendorn, F., Haring, D.A., Maurer, V., Senn, M., Hertzberg, H., 2007. Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticei.* Vet. Parasitol. 146, 123−134.

Hedqvist, H., Mueller-Harvey, I., Reed, J.D., Krueger, C.G., Murphy, M., 2000. Characterisation of tannins and *in vitro* protein digestibility of several *Lotus corniculatus* varieties. Anim. Feed Sci. Technol. 87, 41-56.

Heering, H., Reed, J.D., Hanson, J., 1996. Differences in *Sesbania sesban* accessions in relation to their phenolic concentration and HPLC fingerprints. J. Sci. Food Agric. 71, 92-98.

Hernández-Orduño, G., Torres-Acosta, J.F.J., Sandoval-Castro, C., Aguilar- Caballero, A.J., Reyes-Ramírez, R.R., Hoste, H., Calderón-Quintal, J.A., 2008. *In vitro* anthelmintic effect of *Acacia gaumeri, Havardia albicans* and Quebracho tannin extracts on a Mexican strain of *Haemonchus contortus* L3 larvae. Trop. Subtrop. Agroecosyst. 8, 191−197.

Hernández-Villegas, M.M., Borges-Argaez, R., Rodriguez-Vivas, R.I., Torres-Acosta, J.F.J., Mendez-Gonzalez, M., Caceres-Farfan, M., 2011. Ovicidal and larvicidal activity of crude extracts of *Phytolacca icosandra* against *Haemonchus contortus.* Vet. Parasitol. 179, 100-106.

Hernández-Villegas, M.M., Borges-Argaez, R., Rodriguez-Vivas, R.I., Torres-Acosta, J.F.J., Mendez-Gonzalez, M., Caceres-Farfan, M., 2012. *In vivo* anthelmintic activity of *Phytolacca icosandra* against *Haemonchus contortus* in goats. Vet. Parasitol. 189, 284-290.

Holderness, J., Jackiw, L., Kimmel, E., Kerns, H., Radke, M., Hedges, J.F., Petrie, C., McCurley, P., Glee, P.M., Palecanda, A., Jutila, M.A., 2007. Select plant tannins induce IL-2Rα up-regulation and augment cell division in γδ T cells. J. Immunol. 179, 6468-6478.

Holmes, P.H., 1993. Interactions between parasites and animal nutrition - the veterinary consequences. Proc. Nut. Soc. 52, 113-120.

Hoste, H., Chartier, C., 1993. Comparison of the effects on milk production of concurrent infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in high- and low-producing dairy goats. Am. J. Vet. Res. 54, 1886–1893.

Hoste, H., Huby, F., Mallet, S., 1997. Strongyloses gastrointestinales des ruminants: conséquences physiopathologiques et mécanismes pathogéniques. Le Point Vétérinaire. 28, 53–59.

Hoste, H., Chartier, C., 1998. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats: consequences on milk production. Vet. Parasitol. 74, 43-54.

Hoste, H., 2001. Adaptive physiological processes in the host during gastrointestinal parasitism. Int. J. Parasitol. 31, 231-244.

Hoste, H., Torres-Acosta, J.F., Paolini, V., Aguilar-Caballero, A., Etter, E., Lefrileux, Y., Chartier, C., Broqua, C., 2005. Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. Small Ruminant Res. 60, 141–151.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M., Hoskin, S., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol, 22, 253–261.

Hoste, H., Brunet, S., Paolini, V., Bauhuaud, D., Chaveau, S., Fouraste, I., Lefrileux, Y., 2009. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the southern part of France. In: Papachristou, T.G., Parissi, Z.M., Ben Salem, H., Morand-Fehr, P. (Eds.), Nutritional and Foraging Ecology of Sheep and Goats. Zaragoza: CIHEAM/FAO/NAGREF. CIHEAM/FAO/NAGREF, Zaragoza, pp. 431–436.

Hoste, H., Sotiraki, S., Landau, S.Y., Jackson, F., Beveridge, I., 2010. Goat nematode interactions: Think differently! Trends Parasitol. 36, 376-381.

Hoste, H., Torres-Acosta, J.F.J., 2011. Non chemical control of helminths in ruminants: Adapting solutions for changing worms in a changing world. Vet. Parasitol. 180, 144-154.

Hoste, H., Martinez-Ortiz-de-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infection. Vet. Parasitol. 186, 18–27.

Hotez, P.J., Bethony, J., Bottazzi, M. E., Brooker, S., Diemert, D., Loukas, A. 2006. New technologies for the control of human hookworm infection. Trends Parasitol. 22, 327-331.

Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J., Coop, R.L., 2003. Is the allocation of metabolisable protein prioritised to milk production rather than to immune function in *Teladorsagia circumcincta* infected lactating ewes? Int. J. Parasitol. 33, 327–338.

Houdijk, J.G.M., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2005. Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcta* in sheep. Vet. Parasitol. 129, 105-117.

Houdijk, J.G.M., Kyriazakis, I., Kidane, A., Athanasiadou, S., 2012. Manipulating small ruminant parasite epidemiology through the combination of nutritional strategies. Vet. Parasitol. 186, 38-50.

Hoveland, C.S., Windham, W.R., Boggs, D.L., Durham, R.G., Calvert, G.V., Newsome, J.F., Dobson, F.W., Owsley, M., 1990. Sericea lespedeza production in Georgia. Research Bulletin 393. Georgia Agricultural Experiment Station.

Huemmer, W., Schreier, P. 2008. Analysis of proanthocyanidins. Mol. Nutr. Food Res. 25, 1381-1398.

Hunter, A.R., MacKenzie, G., 1982. The pathogenesis of a single challenge dose of *Haemonchus contortus* in lambs under six months of age. J. Helminthol. 56, 135-144.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003. Can animals use foraging behavior to combat parasites? Proc. Nutr. Soc. 62, 361–370.

Israf, D.A., Coop, R.L., Stevenson, L.M., Jones, D.G., Jackson, F., Jackson, E., MacKellar, A., Huntley, J.F., 1996. Dietary protein influences upon immunity to *Nematodirus battus* infection in lambs. Vet. Parasitol. 61, 273 286.

Israf, D.A., Zainal, M.J., Ben-Gheshir, M.A., Rasedee, A., Sani, R.A., Noordin, M.M., 1998. Dietary protein influences on regulation of *Haemonchus contortus* populations in Dorsimal lambs. J. Helminthol. 72, 143-146.

Jackson, F., Hoste, H., 2010. *In vitro* methods for the primary screening of plant products for direct activity against ruminant gastrointestinal nematodes. In: Vercoe, P.E.; Makkar, H.P.S.; Schlink, A.C. (Eds.), In vitro screening of Plant Resources for Extra Nutritional Attributes in Ruminants: Nuclear and Related Methodologies, FAO/IAEA Springer Edition. pp 24-45.

Jackson, F., Varady, M., Bartley, D.J., 2012. Managing anthelmintic resistance in goats – Can we learn lessons from sheep? Small Rum. Res. 103, 3–9.

Jones, L.A., Houdijk, J.G.M., Sakkas, P., Bruce, A.D., Mitchell, M., Knox, D.P., Kyriazakis, I., 2011. Dissecting the impact of protein versus energy host nutrition on the expression of immunity to gastro intestinal parasites during lactation. Int. J. Parasitol. 41, 711-719.

Joshi, B.R., Kommuru, D.S., Terrill, T.H., Mosjidis, J.A., Burke, J.M., Shakya, K.P., Miller, J.E., 2011. Effect of feeding sericea lespedeza leaf meal in goats experimentally infected with *Haemonchus contortus*. Vet. Parasitol. 178, 192–197.

Juhnke, J., Miller, J., Hall, J.O., Provenza, F.D., Villalba, J.J., 2012. Preference for condensed tannins by sheep in response to challenge infection with *Haemonchus contortus*. Vet. Parasitol. 188, 104-114.

Kabasa, J.D., Opuda-Asibo, J., Ter Meulen, U., 2000. The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. Trop. Anim. Health Prod. 32, 73–86.

Kahiya, C., Mukaratirwa, S., Thamsborg, S.M., 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. Vet. Parasitol. 115, 265–274.

Kahn, L.P., 2003. Regulation of resistance and resilience of periparturient ewes to infection with gastrointestinal nematode parasites by dietary supplementation. Aust. J. Exp. Agr*.* 43, 1477-1486.

Kahn, L.P., Knox, M.R., Gray, G.D., Lea, J.M., Walkden-Brown, S.W., 2003. Enhancing immunity to nematode parasites in single bearing Merino ewes through nutrition and genetic selection. Vet. Parasitol. 112, 211- 225.

Kambara, T., McFarlane, R.G., Abell, T.J., McAnulty, R.W., Sykes A.R., 1993. The effect of age and dietary protein on immunity and resistance in lambs vaccinated with *Trichostrongylus colubriformis*. Int. J. Parasitol. 23, 471–476.

Kates, K.C., Allen, R.W., Wilson, G. I., 1962. Effects of two diets on experimental haemonchosis in lambs. J. Parasitol. 48, 865-870.

Katiki, L.M., Ferreira, J.F.S., Gonzalez, J.M., Zajac, A.M., Lindsay, D.S., Chagas, A.C.S., Amarante, A.F.T., 2013. Anthelmintic effect of plant extracts containing condensed and hydrolyzable tannins on *Caenorhabditis elegans*, and their antioxidant capacity. Vet. Parasitol. 192, 218–227.

Kenyon, F., Jackson, F. 2012. Targeted flock/herd and individual ruminant treatment approaches. Vet. Parasitol. 186, 10-17.

Khan, F.A., Sahoo, A., Sonawane, G.G., Karim, S.A., Dhakad, A.K., Pareek, A.K., Tropathi, B.N., 2012. Effect of dietary protein on responses of lambs to repeated *Haemonchus contortus* infection. Liv. Sci. 150, 143-151.

Kilmister, R.L., Faulkner. P., Downey, M.O., Darby, S.J., Falconer. R.J. 2015. The complexity of condensed tannin binding to bovine serum albumin –an isothermal titration calorimetry study. Food Chem. 190, 173–178.

Klongsiriwet, C., Quijada, J., Williams, A.R., Mueller-Harvey, I. , Williamson, E.M., Hoste, H.. 2015. Synergistic inhibition of *Haemonchus contortus* exsheathment by flavonoid monomers and condensed tannins. Int. J. Parasitol.: Drugs Drug Resist. 5, 127-134.

Knox, M., Steel, J., 1996. Nutritional enhancement of parasite control in small ruminant production systems in developing countries of Southeast Asia and the Pacific. Int. J. Parasitol. 26, 963–970.

Knox, M., 2003.Impact of non-protein nitrogen supplements on nematode infected sheep. Austr. J. Exp. Agric. 43, 1463-1468.

Knox, M.R., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. Vet. Parasitol. 139, 385–393.

Kommuru, D.S., Barker, T., Desai, S., Burke, J.M., Ramsay, A., Mueller-Harvey, I., Miller, J.E., Mosjidis, J.A., Kamisetti, N., Terrill, T.H., 2014. Use of pelleted sericea lespedeza (*Lespedeza cuneata*) for natural control of coccidia and gastrointestinal nematodes in weaned goats. Vet. Parasitol. 204, 191–198.

Kommuru, D.S., Whitley, N.C., Miller, J.E., Mosjidis, J.A., Burke, J.M., Gujja, S., Mechineni, A., Terrill, T.H., 2015. Effect of sericea lespedeza leaf meal pellets on adult female *Haemonchus contortus* in goats. Vet. Parasitol. 207, 170-175.

Kotze, A.C., O'Grady, J., Emms, J., Toovey, A.F., Hughes, S., Jessop, P., Bennell, M., Vercoe, P.E., Revell, D.K., 2009. Exploring the anthelmintic properties of Australian native shrubs with respect to their potential role in livestock grazing systems. Parasitology 136, 1065-1080.

Kumarasingha, R., Palombo, E.A., M. Bhave, M., Yeo, T.C., Lim D.S.L, Tu, C.L., Shaw J.M., Boag P.R., 2014. Enhancing a search for traditional medicinal plants with anthelmintic action by using wild type and stress reporter *Caenorhabditis elegans* strains as screening tools. Int. J. Parasitol., 44, 291-298.

Landau, S., Azaizeh, H., Muklada, H., Glasser, T., Ungar, E.D., Baram, H., Abbas, N., Markovics, A., 2010. Anthelmintic activity of *Pistacia lentiscus* foliage in two Middle Eastern breeds of goats differing in their propensity to consume tannin-rich browse. Vet. Parasitol. 173, 280-286.

Lange, K.C., Olcott, D.D., Miller, J.E., Mosjidis, J.A., Terrill, T.H., Burke, J.M., Kearney, M.T., 2006. Effect of sericea lespedeza (*Lespedeza cuneata*) fed as hay, on natural and experimental *Haemonchus contortus* infections in lambs. Vet. Parasitol. 141, 273-278.

Le Jambre, L.F., 1995. Relationship of blood loss to worm numbers, biomass and egg production in *Haemonchus* infected sheep. Int. J. Parasitol. 25, 269-273.

Leng, R.A., 1991. Optimising herbivore nutrition. In: Ho, Y.W., Wong, H.K., Abdullah, N., Tajuddin, Z.A. (Eds.), Recent Advances on the Nutrition of Herbivores, Proceedings of the 3rd International Symposium on the Nutrition of Herbivores, Pulau Pinang, Malaysia, July 1991. Malaysian Society for Animal Production, UPM, Serdang, Malaysia, pp. 269–281.

Leyva, V., Henderson, A.E., Sykes, A. R., 1982. Effect of daily infection with *Ostertagia circumcincta* larvae on feed intake, milk production and wool growth in sheep. J. Agric. Res. 99, 249-259.

Li, C., Leverence, R., Trombley, J.D., Xu, S., Yang, J., Tian, Y., Reed, J.D., Hagerman, A.E., 2010. High molecular weight persimmon (*Diospyros kaki* L.) proanthocyanidin: a highly galloylated, A-linked tannin with an unusual flavonol terminal unit, myricetin. J. Agric. Food Chem. 58, 9033–9042.

Lopez, J., Ibarra, O.F., Canto, G.J., Vasquez, C.G., Tejada, Z.I., Shimada, A., 2005. *In vitro* effect of condensed tannins from tropical fodder crops against eggs and larvae of the nematode *Haemonchus contortus*. J. Food Agric. Env. 3, 191-194.

Lorenz, M. M., Udén, P., 2011. Influence of formic acid and dry matter on protein degradation in the tanniniferous legume sainfoin. Anim. Feed Sci. Tech. 164, 217–224.

Louvandini, H., Veloso, C.F.M., Paludo, G.R., Dell'Porto, A., Gennari, S.M., McManus, C.M., 2006. Influence of protein supplementation on the resistance and resilience on young hair sheep naturally infected with gastrointestinal nematodes during rainy and dry seasons. Vet. Parasitol. 137, 103–111.

Lowther, W.L., Manley, T.R., Barry, T.N., 1987. Condensed tannin concentrations in *Lotus corniculatus* and *Lotus pedunculatus* cultivars grown under low soil fertility conditions. N. Z. J. Agric. Res. 30, 23-25.

Luginbuhl, J-M., Glennon, H.M., Miller, J.E., Terrill, T.H., 2013. Grazing and pasture management. *Proceedings of the American Consortium for Small Ruminant Parasite Control 10th Anniversary Conference*, May 20-22, 2013, Fort Valley, GA, pages 63-67.

Lüscher, A., Mueller-Harvey, I., Soussana, J.F., Rees, R.M., Peyraud, J.L., 2014. Potential of legume-based grassland-livestock systems in Europe. Grass For. Sci. 69, 206-228.

Lyman, T.D., Provenza, F.D., Villalba, J.J., 2008. Sheep foraging behaviour in response to interactions among alkaloids, tannins and saponins. J. Sci. Food Agric. 88, 824–831.

MacRae, J.C., 1993. Metabolic consequences of intestinal parasitism. Proc. Nutr. Soc. 52, 121–130.

Macarthur, F.A., Kahn, L.P., Windon, R.G., 2013a. Immune response of twin-bearing Merino ewes when infected with *Haemonchus contortus*: effects of fat score and prepartum supplementation. Livest. Sci. 157, 568-576.

Macarthur, F.A., Kahn, L.P., Windon, R.G., 2013b. Regulating maternal production of twin-bearing Merino ewes through fat score and prepartum supplementation when infected with *Haemonchus contortus*. Livest. Sci. 157, 442-451.

Magaya, A., Mukaratirwa, S., Willingham, A.L., Kyvsgaard, N., Thamsborg, S.M., 2000. Effects of anthelmintic treatment and feed supplementation on grazing Tuli weaner steers naturally infected with gastrointestinal nematodes. J. S. Afr Vet. Ass. 71, 31-37.

Mancilla-Leytón, J.M., Joffre, R., Vicente, A.M., 2014. Effect of grazing and season on the chemical composition of Mediterranean shrub species in Donana Natural Park, Spain. J. Arid Environm. 108, 10-18.

Manolaraki, F., 2011. Propriétés anthelminthiques du sainfoin (*Onobrychis viciifoliae*): Analyse des facteurs de variations et du rôle des composés phénoliques impliqués. 21st Jan 2011. INP Toulouse.

Manolaraki, F. Sotiraki, S., Stefanakis, A., Skampardonis, V., Volanis, M., Hoste, H., 2010. Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. Parasitology, 137, 685-696.

Marie-Magdeleine, C., Mahieu, M., Philibert, L., Despois, P., Archimède, H., 2010. Effect of cassava (*Manihot esculenta*) foliage on nutrition, parasite infection and growth of lambs. Small Ruminant Res. 93, 10–18.

Marie-Magdeleine, C., Udino, L., Philibert, L., Bocage, B., Archimede, H., 2014. *In vitro* effects of *Musa x paradisiaca* extracts on four developmental stages of *Haemonchus contortus.* Res. Vet. Sci. 96, 127-132.

Marley, C.L., Cook, R., Barrett, J., Keatinge, R., Lampkin, N.H., 2006. The effects of birdsfoot trefoil (*Lotus corniculatus*) and chicory (*Cichorium intybus*) when compared with perennial ryegrass

(*Lolium perenne*) on ovine gastrointestinal parasite development, survival and migration. Vet. Parasitol. 138, 280-290.

Martínez-Ortiz-de-Montellano, C., Vargas-Magaña, J.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Cob-Galera, L., May-Martínez, M., Miranda-Soberanis, R., Hoste, H., Cámara-Sarmiento, R., Torres-Acosta, J.F.J., 2007. Combining the effects of supplementary feeding and copper oxide needles for the control of gastrointestinal nematodes in browsing goats. Vet. Parasitol. 146, 66−76.

Martínez-Ortíz-De-Montellano, C., Vargas-Magana, J.J., Canul-Ku, H.L., Miranda-Soberanis, R., Capetillo-Leal, C., Sandoval-Castro, C.A., Hoste, H., Torres-Acosta, J.F.J., 2010. Effect of a tropical tannin-rich plant, *Lysiloma latisiliquum*, on adult populations of *Haemonchus contortus* in sheep. Vet. Parasitol. 172, 283-290.

Martínez-Ortíz-de-Montellano, C., Arroyo-López, C., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2013. Scanning electron microscopy of *Haemonchus contortus* exposed to tannin-rich plants under *in vivo* and *in vitro* conditions. Exp. Parasitol. 133, 281−286.

Martínez-Ortiz-de-Montellano, C., Hoste, H., Torres Acosta, J.F.J., Fourquaux, I., 2015. Scanning and Transmission electron microscopy of *Haemonchus contortus* exposed to tannin-rich materials in goats. 16th Int. Scientific Congress 2015. National Center for Scientific Research. La Habana, Cuba. EIN-O-015.

Marume, U., Chimonyo, M., Dzama, K. 2012. Influence of dietary supplementation with *Acacia karoo* on experimental haemonchosis in indigenous Xhosa lop-eared goats of South Africa. Livest. Sci. 144, 132-139.

Max, R.A., Kassuku, A.A., Kimambo, A.E., Mtenga, L.A., Wakelin, D., Buttery, P.J., 2009. The effect of wattle tannin drenches on gastrointestinal nematodes of tropical sheep and goats during experimental and natural infections. J. Agric. Sci. (Camb.) 147, 1−8.

Max, R.A., 2010. Effect of repeated wattle tannin drenches on worm burdens, faecal egg counts and egg hatchability during naturally acquired nematode infections in sheep and goats. Vet. Parasitol. 169, 138−143.

Mbugua, D.M., Kiruiro, E.M., Pell, A.N., 2008. In vitro fermentation of intact and fractionated tropical herbaceous and tree legumes containing tannins and alkaloids. Anim. Feed Sci. Technol. 146, 1−20.

Mc Clure, S.J., 2003. Mineral nutrition and its effects on gastro intestinal immune function of sheep. Aust. J. Exp. Agric. 147, 211-218.

Mc Clure, S.J., 2008. How minerals may influence the development and expression of immunity to endoparasites in livestock. Par. Immunol. 30, 89-100.

Mechineni, A., Kommuru, D.S., Gujja, S., Mosjidis, J.A., Miller, J.E., Burke, J.M., Ramsay, A., Mueller-Harvey, I., Kannan, G., Lee, J.H., Kouakou, B., Terrill, T.H., 2014. Effect of fall-grazed sericea lespedeza (*Lespedeza cuneata*) on gastrointestinal nematode infections, skin and carcass microbial load, and meat quality of growing goats. Vet. Parasitol. 204, 221−228.

Méndez-Ortíz, F.A., Sandoval-Castro, C.A., Torres-Acosta, J.F.J., 2012. Short term consumption of *Havardia albicans* tannin rich fodder by sheep: Effects on feed intake, diet digestibility and excretion of *Haemonchus contortus* eggs. Anim. Feed Sci. Tech. 176, 185-191.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Technol. 106, 3−19.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81, (E suppl 2) 102−109.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing whether goats. Small Ruminant Res. 51, 279−283.

Min, B.R., Hart, S.P., Miller, D., Tomita, G.M., Loetz, E., Sahlu, T., 2005. The effect of grazing forage containing condensed tannins on gastrointestinal parasite infection and milk composition in Angora does. Vet. Parasitol*.* 130, 105−113.

Min, B.R., Solaiman, S., Ramsay, A., Terrill, T.H., Mueller-Harvey, I., 2015a. The effects of tannin-containing ground pine bark diet upon nutrient digestion, nitrogen balance, and mineral retention in meat goats. J. Anim. Sci. Biotechnol. 6, 25 (DOI: 10.1186/s40104-015-0020-5).

Min, B.R., Wilson, E.A., Solaiman, S., Miller, J., 2015b. Effects of condensed tannin-rich pine bark diet on experimentally infected with *Haemonchus contortus* in meat goats. Int. J. Vet. Health Sci. Res. 3, 49-57.

Minho, A.P., Bueno, I.C.S., Louvandini, H., Jackson, F., Gennari, S.M., Abdalla A.L., 2007. Effect of *Acacia molissima* tannin extract on the control of gastrointestinal parasites in sheep. An. Feed Sci. Technol. 147, 172-181.

Minnée, E.M.K., Woodward, S.L., Waghorn, G.C., Laboyrie, P.G., 2002. The effect of ensiling forage legumes on condensed tannins. Agronomy N.Z. 32, 117−119.

Molan, A.L., Meagher, L.P., Spencer, P.A., Sivakumaran, S., 2003a.Effect of flavan-3-ols in vitro hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. Int. J. Parasitol. 33, 1691 – 1698.

Molan, A.L., Duncan, A.J., Barry, T.N., McNabb, W.C., 2003b. Effect of condensed tannins and crude sesquiterpene lactones extracted from chicory on the motility of larvae of deer lungworms and gastrointestinal nematodes. Parasitol. Int. 52, 209–218.

Molan, A.L., Alexander, R., Bookes, I.M., McNabb, W.C., 2004. Effects of sulla condensed tannins on the degradation of ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) and on the viability of three sheep gastrointestinal nematodes *in vitro*. J. Anim. Vet. Adv*.* 3, 165-174.

Molan, A.L., Sivakumaran, S., Spencer, P.A., Meagher, L.P., 2004b. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in vitro. Res Vet. Sci*. 77*, 239-243.

Molan, A.-L., Faraj, A.M., 2010. The effects of condensed tannins extracted from different plant species on egg hatching and larval development of *Teladorsagia circumcincta* (Nematoda: Trichostrongylidae). Folia Parasitol. 57, 62-68.

Moore, D.A., Terrill, T.H., Kouakou, B., Shaik, S.A., Mosjidis, J.A., Miller, J.E., Vanguru, M., Kannan, G., Burke, J.M., 2008. The effects of feeding sericea lespedeza hay on growth rate of goats naturally infected with gastrointestinal nematodes. J. Anim. Sci*.* 86, 2328-2337.

Moreno-Gonzalo, J., Ferre, I., Celaya, R., Frutos, P., Ferreira, L.M.M., Hervás, G., García, U., Ortega-Mora, L.M., Osoro, K., 2012. Potential use of heather to control gastrointestinal nematodes in goats. Small Ruminant Res. 103, 60–68.

Moreno-Gonzalo J., Manolaraki F., Frutos P., Hervás G., Celaya R., Osoro K., Ortega-Mora L.M., Hoste H., I. Ferre I. 2013a. In vitro effect of heather extracts on *Trichostrongylus colubriformis* eggs, larvae and adults. Vet. Parasitol. 197, 586-594.

Moreno-Gonzalo, J., Manolaraki, F., Frutos, P., Hervas, G., Celaya, R., Osoro, K., Ortega-Mora, L.M., Hoste, H., Ferre, I., 2013b. *In vitro* effect of heather (Ericaceae) extracts on different development stages of *Teladorsagia circumcincta* and *Haemonchus contortus*. Vet. Parasitol. 197, 235-243.

Moreno-Gonzalo, J., Osoro, K., García, U., Frutos, P., Celaya, R., Ferreira, L.M.M., Ortega-Mora, L.M., Ferre, I., 2014. Anthelmintic effect of heather in goats experimentally infected with *Trichostrongylus colubriformis.* Parasitol Res. 113, 693-699.

Mosjidis, J.A., Terrill, T.H., 2013. Sericea lespedeza. *Proceedings of the American Consortium for Small Ruminant Parasite Control 10th Anniversary Conference*, May 20-22, 2013, Fort Valley, GA, p 49-52.

Mueller-Harvey, I., 2001. Analysis of hydrolysable tannins. Anim. Feed Sci. Technol. 91, 3-20.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. J. Sci. Food Agric. 86, 2010-2037.

Mupeyo, B.T.N., Pomroy, W.E., Ramírez-Restrepo, C.A., López-Villalobos, N., Pernthaner, A., 2011. Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. Anim. Feed Sci. Technol. 164, 8–20.

Murare, U., Chimonyo, M, Dzama, K., 2012. Influence of dietary supplementation with *Acacia karroo* on experimental haemonchosis in indigenous Xhosa lop-eared goats of South Africa. Livest. Sci. 144, 132-139.

Ncube, S., Mpofu, D., 1994. The nutritive value of wild fruits and their use as supplements to veld hay. Zimbabwe J. Agric. Res. 32, 71–77.

Nguyen, T.M., Binh, D.V., Ørskov, E.R., 2005. Effect of foliages containing condensed tannins and on gastrointestinal parasites. Anim. Feed Sci. Technol. 121, 77–87.

Nichols, C., 1988. Endoscopy, physiology and bacterial flora of sheep infected with abomasal nematodes. Diss. Abstr. Int. 49, 8B.

Nichols, C.D., Hayes, P.R., Lee, D.L., 1987. Physiological and microbiological changes in the abomasum of sheep infected with large doses of *Haemonchus contortus.* J. Comp. Pathol. 97, 299-308.

Niezen, J.H., Waghorn, T.S., Charleston, W.A.G., Waghorn, G.C., 1995. Growth and gastrointestinal nematode parasitism in lambs grazing either lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. J. Agric. Sci. 125, 281–289.

Niezen, J.H., Waghorn, G.C., Charleston, W.A.G., 1998. Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed lotus (*Lotus pedunculatus*) or perennial ryegrass (*Lolium perenne*). Vet. Parasitol. 78, 13–21.

Niezen, J.H., Waghorn, G.C., Graham, T., Carter, J.I., Leathwick, D.M., 2002. The effect of diet fed to lambs on subsequent development of *Trichostrongylus colubriformis* larvae *in vitro* and on pasture. Vet. Parasitol. 105, 269–283.

Nnadi, P.A., Kamalu, T.N., Onah, D.N., 2007. The effect of dietary protein supplementation on the pathophysiology of *Haemonchus contortus* infection in West African Dwarf goats. Vet. Parasitol. 148, 256–261.

Nnadi, P.A., Kamalu, T.N., Onah, D.N., 2009. The effect of dietary protein on the productivity of West African Dwarf (WAD) goats infected with *Haemonchus contortus*. Vet. Parasitol. 161, 232–238.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. Vet. Parasitol. 182, 213-220.

Novobilský, A., Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Enemark H.L., Mueller-Harvey, I., Thamsborg, S.M., 2013. *In vitro* effects of extracts and purified tannins of sainfoin (*Onobrychis viciifolia*) against cattle nematodes. Vet. Parasitol. 196, 532–537.

O'Sullivan, B.M., Donald, A.D., 1973. Responses to infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in ewes of different reproductive status. Int. J. Parasitol. 3, 521-530.

Ojeda-Robertos, N., Manolaraki, F., Theodoridou, K., Aufrère, J., Halbwirth, H., Stich, K., Regos, I., Treutter, D., Mueller-Harvey, I., Hoste, H., 2010. The anthelmintic effect of sainfoin (silage, hay, fresh) and the role of flavonoid glycosides. In: EAAP Meeting Heraklion, Greece 20–24th August 2013, p. 73.

Oliveira, L.M.B., Macedo, I.T.F., Vieira, L.S., Camurca-Vasconcelos, A.L.F., Tome, A.R., Sampaio, R.A., Louvandini, H., Bevilaqua, C.M.L., 2013. Effects of *Mimosa tenuiflora* on larval establishment of *Haemonchus contortus* in sheep. Vet. Parasitol. 196, 341-346.

Omura, S., Miyadera, H., Ui, H., Shiomi, K., Yamaguchi, Y., Masuma, R., Nagamitsu, T., Takano, D., Sunazuka, T., Harder, A., Kölbl, H., Namikoshi, M., Miyoshi, H., Sakamoto, K., Kita, K., 2001. An anthelmintic compound, nafuredin, shows selective inhibition of complex I in helminth mitochondria. Proc. Natl. Acad. Sci. USA 98, 60–62.

Orians, C.M., Griffiths, M.E., Roche, B.M., Fritz, R.S., 2000. Phenolic glycosides and condensed tannins in *Salix sericea*, *S. eriocephala* and their F1 hybrids: not all hybrids are created equal. Biochem. Syst. Ecol. 28, 619–632.

Osborne, N.J.T., McNeill, D.M., 2001. Characterisation of *Leucaena* condensed tannins by size and protein precipitation capacity. J. Sci. Food Agric. 81, 1113–1119.

Ortega-Reyes, L., 1985. Composición química y digestibilidad de la dieta de ovinos Pelibuey bajo condiciones de libre pastoreo en un henequenal de Yucatán. Tec. Pec. Mex. 48, 17–23.

Paolini, V., Frayssines, A., De-La-Farge, F., Dorchies, Ph., Hoste, H., 2003a. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and T*eladorsagia circumcincta* in goats. Vet. Res. 34, 331–339.

Paolini, V., Bergeaud, J.P., Grisez, C., Prevot, F., Dorchies, P., Hoste, H., 2003b. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. Vet. Parasitol. 113, 253–261.

Paolini, V., Dorchies Ph., Hoste H., 2003c. Effects of sainfoin hay on gastrointestinal infection with nematodes in goats. Vet. Rec. 152, 600-601.

Paolini, V., Fouraste, I., Hoste, H., 2004. *In vitro* effects of three woody plant and sainfoin extracts on two parasitic stage of three parasitic nematode species. Parasitology, 129, 69 –77.

Paolini V., Prevot F., Dorchies Ph., Hoste, H., 2005a. Lack of effects of quebracho and sainfoin hay on incoming third stage larvae of *Haemonchus contortus* in goats. Vet. J. 170, 260-263.

Paolini, V., De-La-Farge, F., Prevot, F., Dorchies, Ph., Hoste, H., 2005b. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. Vet. Parasitol. 127, 277–283.

Papagiannopoulos, M., Wollseifen, H.R., Mellenthin, A., Haber, B., Galensa, R., 2004. Identification and quantification of polyphenols in carob fruits (*Ceratonia siliqua* L.) and derived products by HPLC-UV-ESI/MSn. J. Agric. Food Chem. 52, 3784–3791.

Pathak, A.K., Dutta, N., Banerjee, P.S., Pattanaik, A.K., Sharma, K., 2013. Influence of dietary supplementation of condensed tannins through leaf meal mixture on intake, nutrient utilization and performance of *Haemonchus contortus* infected sheep. Asian Australas. J. Anim. Sci. 26, 1446-1458.

Parkins, J.J., Holmes, P.H., 1989. Effects of gastrointestinal helminth parasites on ruminant nutrition. Nutr. Res. Rev. 2, 227– 246.

Phengvichith, V., Ledin, I., 2007. Effect of diet in energy and protein on growth, carcasses characteristics and parasite resistance in goats. Trop. Anim. Health Prod. 39, 59–70.

Poppi, D. P., Sykes, A.R., Dynes, R.A., 1990. The effect of endoparasitism on host nutrition - the implications for nutrient manipulation. Proc. NZ Soc. Anim. Prod. 50, 237-243.

Preston, J.M., Allonby, E.W., 1979. The influence of breed on the susceptibility of sheep to *Haemonchus contortus* infection in Kenya. Res. Vet. Sci. 26, 134-139.

Provenza, F.D., Villalba, J.J., Dziba, L.E., Atwood, S.B., Banner, R.E., 2003. Linking herbivore experience, varied diets, and plant biochemical diversity. Small Ruminant Res. 49, 257–274.

Quijada, J., Fryganas, C., Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., Hoste, H., 2015. Anthelmintic activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from small ruminants are influenced by structural features of condensed tannins. J. Agric. Food Chem. 63, 6346-6354.

Quijada, J., Drake, C., Gaudin, E., El-Korso R., Hoste, H., Mueller-Harvey I., 2015. Tannin content and reactivities in the digestive tract of sheep. submitted for publication.

Rahman, W.A., Collins, G.H., 1991. Changes in liveweight gain and blood constituents in experimental infection of goat-derived compared with a sheep-derived strain of *Haemonchus contortus*. Vet. Parasitol. 38, 145-153.

Rahman, W.A., Collins, G.H., 1992. An association of faecal egg counts and prolactin concentration in sera of periparturient Angora goats. Vet. Parasitol. 43, 85-91.

Ramírez-Restrepo, C.A., Pernthaner, A., Barry, T.N., López-Villalobos, N., Shaw, R.J., Pomroy, W.E., Hein, W.R., 2010. Characterization of immune responses against gastrointestinal nematodes in weaned lambs grazing willow fodder blocks. Anim. Feed Sci. Technol. 99−110.

Ramsay A., Williams A.R., Thamsborg S.M., Mueller-Harvey I. 2015.Galloylated proanthocyanidins from shea (*Vitellaria paradoxa*) meal have potent anthelmintic activity against *Ascaris suum*. Phytochemistry (in press). doi:10.1016/j.phytochem.2015.12.005

Retama-Flores, C., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar-Caballero, A.J., Cámara-Sarmiento, R., Canul-Ku, H.L., 2012. Maize supplementation of Pelibuey sheep in a silvopastoral system: fodder selection, nutrient intake and resilience against gastrointestinal nematodes. Animal, 6, 145−153.

Rhoads, M., Fetterer, R.H., 1996. Developmentally regulated secretion of cathepsin L like cysteine proteases by *Haemonchus contortus*. J. Parasitol. 81, 505-512.

Rhoads, M., Fetterer, R.H., 1996. Extracellular matrix degradation by *Haemonchus contortus*. J. Parasitol. 82, 379-383.

Rios de Alvarez, L., Greer, A.W., Jackson, F., Athanasiadou, S., Kyriazakis, I., Huntley, J.F., 2010. The effect of dietary sainfoin (*Onobrychis viciifolia*) on local cellular responses to *Trichostrongylus colubriformis* in sheep. Parasitology, 135, 1117-1124.

Rocha, R.A., Amarante, A.F.T., Bricarello, P.A., 2004. Comparison of the susceptibility of Santa Ines and Ile de France ewes to nematode parasitism around parturition and during lactation. Small Ruminant Res. 55, 65-75.

Rocha, R.A., Bricarello, P.A., Silva, M.B., Houdijk, J.G.M., Almeida, F.A., Cardia, D.F.F., Amarante, A.F.T., 2011. Influence of protein supplementation during late pregnancy and lactation on the resistance of Santa Ines and Ile de France ewes to *Haemonchus contortus*. Vet. Parasitol. 181, 229−238.

Rochfort, S., Parker, A.J., Dunshea, F.R., 2008. Plant bioactives for ruminant health and productivity. Phytochemistry, 69, 299−322.

Rodriguez-Perez, C., Quirantes-Pine, R., Amessis-Ouchemoukh, N., Madani, K., Seguar-Carretero A., Fernandex-Gutierrez, A., 2013. A metabolite-profiling approach allows the identification of new compounds from *Pistacia lentiscus* leaves. J. Pharmaceut. Biomed. Anal. 77, 167-174.

Romani, A., Pinelli, P., Galardi, C., Mulinacci, N., Tattini, M., 2002. Identification and quantification of galloyl derivatives, flavonoid glycosides and anthocyanins in leaves of P*istacia lentiscus* L., Phytochem. Anal. 13, 79-86.

Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., 2015. Condensed tannins in extracts from European medicinal plants and herbal products. J. Pharm. Biomed. Anal. (submitted*).*

Rowe, J.B., Dargie, J.D., Holmes, P.H., 1982. The effect of haemonchosis and blood loss into the abomasum on N digestion in sheep. Proc. Nutr. Soc. London, 41, 74A.

Sakkas, P., Jones, L.A., Houdijk, J.G.M., Athanasiadou, S., Knox, D.P., Kyriazakis, I., 2011. Dietary protein and supplies differentially affect resistance to parasites in lactating mammals. Br. J. Nutr. 108, 1207-1215.

Sakkas, P., Houdijk, J.G.M., Athanasiadou, S., Knox, D.P., Kyriazakis, I.K., 2012a. Sensitivity of periparturient breakdown of immunity to parasites to dietary protein source. J. An. Sci. 90, 3954-3962.

Sakkas, P., Jones, L.A., Houdijk, J.G.M., Athanasiadou, S., Knox, D.P., Kyriazakis, I.K., 2012b. Leucine and methionine deficiency impairs immunity to gastrointestinal parasites during lactation. Br. J. Nutr. 109, 1-10.

Salman, S.K., Duncan, J.L., 1984. The abomasal histology of worm-free sheep given primary and challenge infections with *Haemonchus contortus.* Vet. Parasitol. 16, 43-54.

Salman, S.K., Duncan, J.L., 1985. Studies on the abomasal pathology of immunized and non-immunized sheep infected with *Haemonchus contortus.* J. Helminthol. 59, 351-359.

Salminen, J.-P., Ossipov, V., Loponen, J., Haukioja, E., Pihlaja, K., 1999. Characterisation of hydrolysable tannins from leaves of *Betula pubescens* by high-performance liquid chromatography−mass spectrometry. J. Chromatogr. A, 864, 283−291.

Salminen, J.-P., Karonen. M., 2011. Chemical ecology of tannins and other phenolics: we need a change in approach. Funct. Ecol. 25, 325-338.

Schofield, P., Mbugua, D.M., Pell, A.N., 2001. Analysis of condensed tannins: a review. Anim. Feed Sci. Technol. 91, 21-40.

Self, R., Eagles, J., Galletti, G.C., Mueller-Harvey, I., Hartley, R.D., Lea, A.G.H., Magnolato, D., Richli, U., Gujer, R., Haslam, E., 1986. Fast atom bombardment mass spectrometry of polyphenols (syn. vegetable tannins). Biomed. Environm. Mass Spectrom. 13, 449-468.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kallu, R.K., Mosjidis, J.A., 2004. Effects of feeding sericea lespedeza hay to goats infected with *Haemonchus contortus*. S. Afr. J. Anim. Sci. 34, 248−250.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kaplan, R.M., Burke, J.M., Mosjidis, J.A., 2006. Sericea lespedeza hay as a natural deworming agent against gastrointestinal nematode infection in goats. Vet. Parasitol. 139, 150−157.

Shaw, K.L., Nolan, J.V., Lynch, J.J., Coverdale, O.R., Gill, H.S., 1995. Effects of weaning, supplementation and gender on acquired immunity to *Haemonchus contortus* in lambs. Int. J. Parasitol. 25, 381-387.

Silanikove, N., Perevolotsky, A., Provenza, F.D., 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. Anim. Feed Sci. Technol. 91, 69-81.

Simpson, H.V., 2000. Pathophysiology of abomasal parasitism: is the host or parasite responsible? Vet. J. 160, 177-191.

Singh, R., Knox, M.R., Leng, R.A., Nolan,J.V.,1995. Aspects of parasite management in goats. In: Gill, H.S. (Ed.), Novel ustralia, 54 pp.

Singh, S., Pathak, A.K., Sharma, R.K., Khan, M., 2015. Effect of tanniferous leaf meal based multi-nutrient blocks on feed intake, hematological profile, immune response, and body weight changes in *Haemonchus contortus* infected goats. Vet. World 8, 572-579.

Sirama, V., Kokwaro, J., Owuor, B., Yusuf, A., Kodhiambo, M., 2015. *In-vitro* anthelmintic activity of *Vernonia amygdalina* Del. (Asteraceae) roots using adult *Haemonchus contortus* worms. Int. J. Pharmacol. Res. 5, 1-7

Sivakumaran, S., Molan, A.L., Meagher, L.P., Kolb, B., Foo, L.Y., Lane, G.A., K.M., 2004. Variation in antimicrobial action of proanthocyanidins from *Dorycnium rectum* against rumen bacteria. Phytochemistry 65, 2485−2497.

Sivakumaran, S., Rumball, W., Lane, G.A., Fraser, K., Foo, L.Y., Yu, M., Meagher, L.P., 2006. Variation of proanthocyanidins in *Lotus* species. J. Chem. Ecol. 32, 1797−1816.

Seng, S., Rodriguez, L., 2001. Foliage from cassava, *Flemingia macrophylla* and bananas compared with grasses as forage sources for goats: effects on growth rate and intestinal. Liv. Res. Rural Dev. 13.

Seng, S., Preston, T.R., 2003. Effect of grass or cassava foliage on growth and nematode parasite infestation in goats fed low or high protein diets in confinement. Liv. Res. Rural Dev. 15.

Sokerya, S., Waller, P.J., Try, P., Hoglund, J. 2009. The effect of long term feeding of fresh and ensiled cassava (*Manihot esculenta*) foliage on gastro intestinal nematode infections in goats. Trop. Anim. Health Prod. 41, 251-258.

Spencer, P., Sivakumaran, S., Fraser, K., Foo, L.P., Lane, G.A., Edwards, P.J.B., Meagher, L.P., 2007. Isolation and characterisation of procyanidins from *Rumex obtusifolius*. Phytochem. Anal. 18, 193−203.

Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Brown, R.H., Mueller-Harvey, I., 2012. Proanthocyanidin diversity in the EU 'HealthyHay' sainfoin (*Onobrychis viciifolia*) germplasm collection. Phytochemistry, 77, 197-208.

Suttle, N.F., Knox, D.P., Angus, K.W., Jackson, F., Coop, R.L., 1992. Effects of dietary molybdenum on nematode and host during *Haemonchus contortus* infection in lambs. Res. Vet. Sci. 52, 230-235.

Suttle, N.F., Jones, D.G. 1989. Recent developments in trace element metabolism and function: trace elements, disease resistance and immune responsiveness in ruminants. Am. Institute Nutr. 1055-1061.

Sykes, A.R., Greer A.W., 2003. Effects of parasitism on nutrition economy of sheep: an overview. Aus J. Exp. Agric. 43, 1393-1398.

Terrill, T.H., Windham, W.R., Hoveland, C, S., Amos, H.E., 1989. Influence of forage preservation method on tannin concentration, intake and digestibility of sericea lespedeza by sheep. Agronomy J. 81, 435-439.

Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N., 1992. Determination of extractible and bound condensed tannin concentrations in forage plants, protein concentrate meals, and cereal grains. J. Sci. Food Agric. 58, 321-329.

Terrill, T.H., Waghorn, G.C., Woolley, D.J., McNabb, W.C., Barry, T.N., 1994. Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep. Br. J. Nutr. 72, 467−477.

Terrill, T.H., Mosjidis, J.A., Moore, D.A., Shaik, S.A., Miller, J.E., Burke, J.M., Muir, J.P., Wolfe, R., 2007. Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. Vet. Parasitol. 146, 117-122.

Terrill, T.H., Soli, F.A., Vanguru, M., Vuggam, A., Shaik, S.A., Mosjidis, J.A., Miller, J.E., Kouakou, B., Burke, J.M., 2008. Effectiveness of sericea lespedeza leaf meal to reduce worm burden in goats. *9th International Conference on Goats*, August 31st to September 5th, 2008, Queretaro, Mexico, p. 258.

Terrill, T.H., Dykes, G.S., Shaik, S.A., Miller, J.E., Kouakou, B., Kannan, G., Burke, J.M., Mosjidis, J.A., 2009. Efficacy of sericea lespedeza hay as a natural dewormer in goats: dose titration study. Vet. Parasitol. 163, 52-56.

Terrill, T.H., Miller, J.E., Burke, J.M., Mosjidis, J.A., Kaplan, R.M., 2012. Experiences with integrated concepts for the control of *Haemonchus contortus* in sheep and goats in the United States. Vet. Parasitol. 186, 28-37.

Theodoridou, K., Aufrère, J., Andueza, D., Le Morvan, A., Picard, F., Stringano, E., Pourrat, J., Mueller-Harvey, I., Baumont, R., 2011. Effect of plant development during first and second growth cycle on chemical composition, condensed tannins and nutritive value of three sainfoin (*Onobrychis viciifolia*) varieties and lucerne. Grass For. Sci. 66, 402–414.

Theodoridou, K., Aufrère, J., Andueza, D., Pourrat, J., Le Morvan, A., Stringano, E., Mueller-Harvey, I., Baumont, R., 2010. Effects of condensed tannins in fresh sainfoin (*Onobrychis viciifolia*) on *in vivo* and *in situ* digestion in sheep. Anim. Feed Sci. Technol. 160, 23-38.

Tibe, O., Meagher, L.P., Fraser, K., Harding, D.R.K., 2011. Condensed tannins and flavonoids from the forage legume sulla (*Hedysarum coronarium*). J. Agric. Food Chem. 59, 9402–9409.

Tibe, O., Pernthaner, A., Sutherland, I., Lesperance, L., Harding, D.R.K., 2012. Condensed tannins from Botswanan forage plants are effective priming agents of γδ T cells in ruminants. Vet. Immunol. Immunopathol. 146, 237-244.

Torres-Acosta, J.F.J., 1999. Supplementary feeding and the control of gastrointestinal nematodes of goats in Yucatán, México. PhD Thesis. The Royal Veterinary College. University of London.

Torres-Acosta, J.F.J., Jacobs, D.E., Aguilar-Caballero, A., Sandoval-Castro, C., May-Martínez, M., Cob-Galera, L.A., 2004. The effect of supplementary feeding on the resilience and resistance of browsing Criollo kids against natural gastrointestinal nematode infections during the rainy season in tropical Mexico. Vet. Parasitol. 124, 217–238.

Torres-Acosta, J.F.J., 2006. The effect of supplementary feeding in browsing Criollo kids and hair sheep naturally infected with gastrointestinal nematodes. In: Sandoval-Castro, C.A., Hovell, F.D.DeB.D., Torres-Acosta, J.F.J., and Ayala-Burgos, A.J. (Eds.). The assessment of intake, digestibility and the roles of secondary compounds. BSAS Publication 34. Nottingham University Press. Pp. 261-278.

Torres-Acosta, J.F.J., Jacobs, D.E., Aguilar-Caballero, A., Sandoval-Castro, C., Cob-Galera, L., May-Martínez, M., 2006. Improving resilience against natural gastrointestinal nematode infections in browsing kids during the dry season in tropical Mexico. Vet. Parasitol. 135, 163–173.

Torres-Acosta J.F.J., Hoste, H., 2008. Alternative or improved methods to limit gastro-intestinal parasitism in grazing / browsing sheep and goats. Small Ruminant Res. 77, 159-173.

Torres-Acosta, J.F.J., Mendoza-de-Gives, P., Aguilar-Caballero, A.J., Cuéllar-Ordaz, J.A., 2012. Anthelmintic resistance in sheep farms: update of the situation in the American continent. Vet. Parasitol. 189, 89–96.

Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., Aguilar-Caballero, A.J., Cámara-Sarmiento, R., Alonso-Díaz, M.A., 2012. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. Small Ruminant Res. 103, 28–40.

Tuominen, A., Toivonen, E., Mutikainen, P., Salminen, J.-P., 2013. Defensive strategies in *Geranium sylvaticum*. Part 1: Organ-specific distribution of water-soluble tannins, flavonoids and phenolic acids. Phytochemistry, 95, 394–407.

Tzamaloukas, O., Athanasiadou, S., Kyriazakis, I., Huntley, J., 2006. The effect of chicory (*Cichorium intybus*) and sulla (*Hedysarum coronarium*) on larval development and mucosal cell responses of growing lambs challenged with *Teladorsagia circumcincta*. Parasitology, 132, 419–426.

Valderrábano, J., Uriarte, J., 2003. Effect of nutrition in early pregnancy on the periparurient relaxation of immunity to gastro intestinal parasitism in prolific ewes. Anim. Sci. 76, 481-489.

Valderrábano, J., Gomez-Ricon, J., Uriarte, J., 2005. Effect of nutritional status and fat reserves on the periparturient immune response to *Haemonchus contortus* infection in sheep. Vet. Parasitol. 141, 122-131.

Valderrábano, J., Calvete, J., Uriarte, J., 2010. Effect of feeding bioactive forages on infection and subsequent development of *Haemonchus contortus* in lamb faeces. Vet. Parasitol. 172, 89–94.

Van-Houtert, M.F.J., Barger, I.A., Steel, J.W., 1995. Dietary protein for young grazing sheep: interactions with gastrointestinal parasitism. Vet. Parasitol. 60, 283–295.

Van-Houtert, M.F.J., Barger, I.A., Steel, J.W., 1996. Supplementary feeding and gastrointestinal nematode parasitism in young grazing sheep. Proc. N.Z. Soc. Anim. Prod 56, 94– 98.

Van-Houtert, M.F.J., Sykes, A.R., 1996. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. Int. J. Parasitol. 26, 1151–1167.

Van Soest, P.J., 1994. Nutritional Ecology of the Ruminant, 2nd ed. Cornell University Press, Ithaca, NY, USA.

Van Wyk, J.A., Bath, G.F. 2002. The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Vet. Parasitol. 33, 509-529.

Vargas-Magaña, J., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Hoste, H., Chan-Pérez, J.I., 2014a. Anthelmintic activity of acetone−water extracts against *Haemonchus contortus* eggs: Interactions between tannins and other plant secondary compounds. Vet. Parasitol. 206, 322−327.

Vargas-Magaña, J.J., Torres-Acosta, J.F.J., Aguilar-Caballero, A.J., Sandoval-Castro, C.A., Hoste, H., Chan-Pérez, J.I., Mathieu, C., Vilarem, G., 2014b. *In vitro* susceptibility to tannin rich extracts differs amongst *Haemonchus contortus* isolates from tropical and temperate regions. In: 13th International Congress of Parasitology, August 10−15, 2014. México City, México.

Villalba, J.J., Provenza, F. D., 2007. Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. Animal, 1, 1360-1370.

Villalba, J.J, Miller, J., Ungar, E., Landau, S.Y., Glendinning, J., 2014. Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism and origins. Parasite, 21, 31-40.

von Son-de Fernex, E., Alonso-Diaz, M.A., Valles-de la Mora, B., Capetillo-Leal, C.M., 2012. *In vitro* anthelmintic activity of five tropical legumes on the exsheathment and motility of *Haemonchus contortus* infective larvae. Exp. Parasitol. 131, 413-418.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. An. Feed Sci. Technol. 147, 116-139.

Wallace, D.S., Bairden, K., Duncan, J.L., Fishwick, G., Gill, E.M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M. J., 1994. The effect of urea supplementation and feeding level on infection with *Haemonchus contortus* in Hampshire Down sheep. Proc. European Assoc. Anim. Prod., Edinburgh, Scotland, U.K., pp. 253.

Wallace, D.S., Bairden, K., Duncan, J.L., Fishwick, G., Gill, M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M.J., 1995. Influence of supplementation with dietary soyabean meal on resistance to haemonchosis in Hampshire down lambs. Res. Vet. Sci. 58, 232−237.

Wallace, D.S., Bairden, K., Duncan, J.L., Fishwick, G., Gill, M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear, M.J., 1996. Influence of soyabean meal supplementation on the resistance of Scottish blackface lambs to haemonchosis. Res. Vet. Sci. 60, 138−143.

Wallace, D.S., Bairden, K., Duncan, J.L., Eckersall, P.D., Fishwick, G., Gill, E.M., Holmes, P.H., McKellar, Q.A., Murray, M., Parkins, J.J., Stear M. J. 1998. The influence of dietary supplementation with urea on resilience and resistance to infection with *Haemonchus contortus.* Parasitology, 116, 67-72.

Wallace, D.S., Bairden, K., Duncan, J.L., Eckersall, P.D., Fishwick, G., Holmes, P.H., McKellar, Q.A., Mitchell, S., Parkins, J.J., Stear, M.J., 1999. The influence of increased feeding on the susceptibility of sheep to infection with *Haemonchus contortus*. Anim. Sci. 69, 457−463.

Walkden-Brown, S.W., Kahn, L.P., 2002. Nutritional modulation of resistance and resilience to gastrointestinal nematode infection. Asian-Austr. J. Anim. Sci. 15, 912−924.

Wang, Y., McAllister, T.A., Acharya, S., 2015. Condensed tannins in sainfoin: composition, concentration and effects on nutritive and feeding value of sainfoin forage. Crop Sci. 55, 13-22.

Waruiru, R.M., Ngotho, J.W., Mutune, M.N., 2004. Effect of urea-molasses block supplementation on grazing weaner goats naturally infected with gastrointestinal nematodes. Onderstepoort J. Vet. Res. 71, 285−289.

Werne, S. Perler, E., Maurer, V., Probst, J., Drewek, A., Hoste, H., Heckendorn, F., 2013. Effect of sainfoin and faba bean on gastrointestinal nematodes in periparturient ewes. Small Ruminant Res. 113, 454-460.

Whitney, T.R, Wildeus, S., Zajac, A.M., 2013. The use of redberry juniper (*Juniperus pinchotii*) to reduce *Haemonchus contortus* fecal egg counts and increase ivermectin efficacy. Vet. Parasitol. 197, 182-188.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014a. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. PLoS ONE 9(5): e97053. doi:10.1371/journal.pone.0097053

Williams, A.R., Ropiak, H.M., Fryganas, C., Desrues, O., Mueller-Harvey, I., Thamsborg, S.M., 2014b. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of*Oesophagostomum dentatum.* Parasites & Vectors 2014b, 7:518.

Williams, A.R., Ramsay, A., Hansen, T.V.A., Ropiak, H.M., Mejer, H., Nejsum, P., Mueller-Harvey, I., Thamsborg, S.M., 2015. Anthelmintic activity of trans-cinnamaldehyde and A- and B-type proanthocyanidins derived from cinnamon (*Cinnamomum verum*). Nature Scientific. Rep. 5:14791 | DOI: 10.1038/srep14791.

Williamson, A.L., Brindley, P.J., Knox, D.P., Hotez, P.J., Loukas, A. 2003. Digestive proteases of blood-feeding nematodes. Trends Parasitol. 19, 417-423.

Wilson, L.L., Merritt, T.L., Rugh, M.C, Thompson, C.E., Rothenbacher, H., 1969. Effects of *Haemonchus contortus* inoculation on growth rate, feed efficiency and haematology of feeder lambs Vet. Med. Small An. Clin. 64, 59-62.

Wright, C., 2015. The effects of phytochemical tannin-containing diets on animal performance and internal parasite control in meat goats. MSc thesis, Tuskegee University, Tuskegee, Alabama, USA.

Xhomfulana, V., Mapiye, C., Chimonyo, M., Marufu, M.C., 2009. Supplements containing *Acacia karroo* foliage reduce nematode burdens in Nguni and crossbred cattle. Anim. Prod. Sci. 49, 646–653.

Yu, F, Bruce, L A., Calder, A G., Milne, E., Coop, R L., Jackson, F., Horgan, G W., MacRae, J. C. 2000. Subclinical infection with the nematode *Trichostrongylus colubriformis* increases gastro-intestinal tract leucine metabolism and reduces availability of leucine for other tissues. J. An. Sci. 78, 380-390.

Zeller, W.E., Ramsay, A., Ropiak, H.M., Fryganas, C., Mueller-Harvey, I., Brown, R.H., Drake, C., Grabber, J.H., 2015a. ^{1}H-^{13}C HSQC NMR spectroscopy for estimating procyanidin/prodelphinidin and *cis*/*trans* flavanol ratios of condensed tannin fractions: correlation with thiolysis. J. Agric. Food Chem. 63, 1967−1973.

Zeller, W.E., Sullivan, M.L., Mueller-Harvey, I., Grabber, J.H., Ramsay, A., Drake, C., Brown, R.H., 2015b. Protein precipitation behavior of condensed tannins from *Lotus pedunculatus* and *Trifolium repens* with different mean degrees of polymerization. J. Agric. Food Chem. 63, 1160−1168.

Zhong, R.Z., Li, H.Y., Sun, H.X., Zhou, D.W., 2014. Effects of supplementation with dietary green tea polyphenols on parasite resistance and acute phase protein response to *Haemonchus contortus* infection in lambs. Vet. Parasitol. 205, 199–207.

Box 1= A BOX OF DEFINITIONS

- **Feeds:** Diets offered to livestock animals in order to cover primarily their nutritional requirements (i.e. macro- and micro-nutrients such as energy, protein/amino acids, fatty acids, vitamins, minerals, etc.) for survival, reproduction and production. However, a feed can also include 'non-nutritional' components (e.g. PSMs).
- **Feedstuff:** Material that can be used by livestock animals to obtain nutrients. These materials can originate from plants (i.e. sorghum grain) or animal by-products (i.e. fishmeal). They can be used as ingredients to combine with other materials to form a feed designed to meet the nutritional requirements of animals. Each feedstuff is characterized by a certain quantity of macro- and micro-nutrients and may also contain secondary plant compounds.
- **Nutrients:** Dietary components that meet the nutritional requirements of the animal and include macro- and micro-nutrients.
- **Nutraceuticals:** Based on the definition by Andlauer and Furst (2002) for medicinal foods. A nutraceutical in veterinary science can be defined as a livestock feed, which combines nutritional value with beneficial effects on animal health. This two-pronged action is considered to stem from the presence of various plant secondary metabolites (PSM) or bioactive compounds (Hoste et al., 2015).
- **Plant secondary metabolites (PSMs):** Plants synthesize primary substances (e.g. cell walls, proteins, lipids, DNA) for their basic functioning. They also produce secondary metabolites to adapt to environmental conditions (plant defense to diseases and aggressors, UV screens, adaptation to physical or chemical stress, etc). These compounds may also have numerous other roles in animal and human health and nutrition and are the focus of much research.
- **Self-medication:** Self-medicative behaviors have been observed when plants that contain natural AHs are selectively consumed by ruminants. This can be classified into two types of feeding behavior: prophylactic and therapeutic behaviors that can aid in controlling intestinal parasites or can provide relief from gastrointestinal disorders (Villalba and Provenza, 2007; Villalba et al., 2014).
- **Resistance:** This refers to the ability of an infected host to regulate nematode populations through immune responses that involve complex mechanisms (Balic et al., 2000). The acquisition of host resistance is progressive and depends on nematode factors (e.g. GIN species, frequency of contact with the GINs) and host factors (e.g. host species and age, genetic, individual factors and host nutrition) (Hoste et al., 2010; Van Houtert and Sykes, 1996). Four effects of host resistance on the biological traits at different stages of the GIN life cycle have been described: i) decrease in the establishment of infective third stage larvae (L3), ii) reduced growth and development of L3, L4 and S5, when established in the host; iii) reduced fertility of adult (female) worm populations and iv) expulsion of established worm populations (Balic et al., 2000).
- **Resilience:** This refers to the ability of an infected host to maintain normal (physiological) function and health as measured by production and pathophysiological parameters when infected with GIN (derived from Albers et al., 1987). If we consider that GIN infection, i.e. H. contortus, can cause additional nutrient requirements, then supplemented animals improve their resilience by using the additional macro- and micro-nutrients from supplementary feeding to reduce the pathophysiological effects of infection, and, if available, some extra nutrients may also enhance growth rate or milk production.

Table 1: *In vitro* methods used to evaluate the anthelmintic activity of CT-containing resources

	Equipment required	Parasite stage	Measured anthelmintic effect	References
EHA (Egg Hatch Assay)	Optical microscope, dry oven	Eggs	Egg hatching disrupted	Chan-Pérez et al., submitted; Hernández-Villegas et al., 2011; Houzangbé-Adote et al., 2005a, 2005b; Molan, 2014; Moreno-Gonzalo et al., 2013a, 2013b; Paolini et al., 2004; Vargas-Magaña et al., 2014
LDIA (Larval Development Inhibition Assay)	Optical microscope, dry oven	Eggs - L3	Development from eggs to L3 delayed/blocked	Athanasiadou et al., 2001; Molan, 2014.
LFIA (Larval Feeding Inhibition Assay)	Fluorescence microscope, dry oven	L1	Feeding of L1 disrupted/blocked	Desrues et al., 2016; Novobilský et al., 2011, 2013.
LMIA (Larval Migration Inhibition Assay)	Optical microscope, dry oven	L3	Decreased motility, paralysis	Alonso-Díaz et al., 2008a, 2008b, 2011; Calderón-Quintal et al., 2010; Hernández-Orduño et al., 2008; Hernandez-Villegas et al., 2011; Hoste et al., 2009; Houzangbé-Adote et al., 2005a, 2005b; Molan, 2014; Molan et al., 2004; Moreno-Gonzalo et al., 2013a, 2013b; Paolini et al., 2004
LEIA (Larval Exsheathment Inhibition Assay)	Optical microscope, dry oven	L3	Exsheathment of L3 delayed/blocked	Alonso-Díaz et al., 2008a, 2008b, 2011; Bahuaud et al., 2006; Novobilský et al., 2011; Quijada et al., 2015.
AMIA (Adult Motility Inhibition Assay)	Stereo microscope, dry oven	L5 or adults	Motility (viability) of worms affected	Hoste et al., 2009; Houzangbé-Adote et al., 2005a, 2005b; Moreno-Gonzalo et al., 2013a, 2013b; Paolini et al., 2004

Table 2: Compared *in vitro* anthelmintic effects of tannin-containing resources against *H. contortus* and other GIN nematodes species. (NS= non significant)

Plant	Botanical family	Host	Kind of CT sample	*H. contortus*	*Te. circumcincta*	*T. colubriformis*	*Ostertagia*	*Cooperia*	EC_{50}	References	Main findings
Temperate legumes											
Lespedeza cuneata	Leguminosae	Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Lotus corniculatus	Leguminosae	Cattle	Extract				x	x	Yes	Novobilský et al., 2011	AH effect against both species
Lotus pedunculatus	Leguminosae	Cattle	Extract				x	x	Yes	Novobilský et al., 2011	AH effect against both species
Onobrychis viciifolia	Leguminosae	Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
		Cattle	Extract				x	x	Yes	Novobilský et al., 2011	AH effect against both species
		Cattle	Extract & Fraction				x	x	No	Novobilský et al., 2013	AH effect against both species, variations between extracts or fractions tested
		Goat	Extract	x	x	x			No	Paolini et al., 2004	AH effect on *T. colubriformis* and *H. contortus* L3, and on abomasal adult worms
		Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
Trifolium repens	Leguminosae	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Sarothamnus scoparius	Leguminosae	Goat	Extract	x		x			No	Bahuaud et al. 2006	No AH effect for both species
				x	x	x				Hoste et al., 2009	AH effect vary according to the specie and stage, thus were significant for *H. contortus* and *T. colubriformis* adult worms but only for *Te. circumcincta* L3
Tropical Legumes											
Acacia pennatula	Leguminosae	Goat	Extract	x		x			No	Alonso-Díaz et al., 2008a, 2008b	AH effect was similar for both parasites
Leucaena leucocephala	Leguminosae	Goat	Extract	x		x			No	Alonso-Díaz et al., 2008a, 2008b	AH effect was similar for both parasites
Lysiloma latisiliquum	Leguminosae	Goat	Extract	x		x			No	Alonso-Díaz et al., 2008a, 2008b	AH effect was similar for both parasites
Piscidia piscipula	Leguminosae	Goat	Extract	x		x			No	Alonso-Díaz et al., 2008a, 2008b	AH effect was similar for both parasites

Continuation… Table 2

Plant	Botanical family	Host	Kind of CT sample	*H. contortus*	*Te. circumcincta*	*T. colubriformis*	*Ostertagia*	*Cooperia*	EC_{50}	References	Main findings
Shrubs/woody plants											
Betula spp	Betulaceae	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Calluna vulgaris	Ericaceae	Goat	Extract	x	x	x			Yes	Moreno-Gonzalo et al., 2013a, 2013b	Showed the higher EC50 of heather's extracts. The AH effect vary according to the parasite stage, for EHA only *T. colubriformis* was affected and it showed the lower EC50 for LEIA also
Camellia sinensis	Theaceae	Sheep	Fraction		x	x			No	Molan et al., 2004	AH effect against both species, *Te. circumcincta* was more susceptible
Castanea sativa	Fagaceae	Goat	Extract	x	x	x			No	Bahuaud et al., 2006	Similar AH effect against *H. contortus* and *T. colubriformis* (L3)
										Hoste et al., 2009	High AH effect vary according to the specie and stage. For adults of *Te. circumcincta* was NS
Corylus avellana	Corylaceae	Cattle	Fraction				x	x		Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Extract	x	x	x			No	Hoste et al., 2009	AH effect against the three species (both L3, adults), only the L3 of *T. colubriformis* was non not significant
			Extract	x	x	x			No	Paolini et al., 2004	AH effect on abomasal species (L3 and adult) and intestinal adult worm, but non on L3
			Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Erica cinerea	Ericaceae	Goat	Extract	x	x	x			Yes	Moreno-Gonzalo et al., 2013a, 2013b	Extract's AH effect vary according to the parasite stage, for EHA only *T. colubriformis.* For LEIA, the lower EC50 were for abomasal species
Erica erigenea	Ericaceae	Goat	Extract	x	x	x			No	Bahuaud et al., 2006	*H. contortus* was more susceptible
										Hoste et al., 2009	*Te. circumcincta* (L3 and adult) was the less susceptible
Erica umbellata	Ericaceae	Goat	Extract	x	x	x			Yes	Moreno-Gonzalo et al., 2013a, 2013b	Extract's AH effect vary according to the parasite stage, for EHA only *T. colubriformis* was affected and it showed the lower EC50 for LEIA also
Fraxinus excelsior	Oleaceae	Goat	Extract	x	x	x			No	Hoste et al., 2009	Similar AH effect on abomasal and intestinal species (L3 only)
Junglans regia	Junglandecea	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)

Continuation... Table 2

Plant	Botanical family	Host	Kind of CT sample	*H. contortus*	*Te. circumcincta*	*T. colubriformis*	*Ostertagia*	*Cooperia*	EC_{50}	References	Main findings
Newboldia laevis	Bignoneaceae	Sheep	Extract	x		x			No	Azando et al., 2011	Similar AH effect on both species (L3)
										Houzangbé-Adote et al 2005a, 2005b	AH effect on L3 on both species but only on *T. colubriformis* adut worms
Pinus radiata	Pinaceae	Sheep	Fraction		x	x			Yes	Molan, 2014	AH effect on both species. *Te. circumcincta* either egg or larvae were more susceptible (lower EC50)
Pinus sylvestris	Pinaceae	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Extract	x		x			No	Bahuaud et al., 2006	Similar AH effect against *H. contortus* and *T. colubriformis* (L3)
			Extract	x	x	x			No	Hoste et al., 2009	*T. colubriformis* was the most susceptible (both L3 and adult). For the two abomasal species, AH effect was significant on *H. contortus* adult worms only
			Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Quercus robur	Fagaceae	Goat	Extract	x	x	x			No	Hoste et al., 2009	AH effect was NS on *T. colubriformis* L3
Ribus nigrum	Grossulariacea	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Ribus rubrum	Grossulariacea	Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Rubus fructicosus	Rosaceae	Goat	Extract	x	x	x			No	Hoste et al., 2009	AH effect was NS just for *H. contortus* L3
Salix spp	Salicaceae	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Schinopsis spp	Anacardiaceae	Sheep	Extract	x	x	*T. vitrinus*			Yes	Athanasiadou et al., 2001	Similar AH effect on the 3 species (EC50 were non significantly different)
Theobroma cacao	Malvaceae	Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Tilia spp	Tiliaceae	Cattle	Fraction				x	x	No	Desrues et al., 2016	AH effect against both species, *O. ostertagi* was more susceptible
		Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Vitelaria paradoxa	Sapotaceae	Goat	Fraction	x		x			Yes	Quijada et al., 2015	AH effect against both species, *H. contortus* was more susceptible (lower EC50)
Zanthoxylum zanthoxuloïdes	Rutaceae	Sheep	Extract	x		x			No	Azando et al., 2011	Similar AH effect on both species
										Houzangbé-Adote et al. 2005a, 2005b	AH effect observed on both species, *H. contortus* (adult) appeared less susceptible

Table 3: Compared *in vivo* anthelmintic efficacy of tannin-containing resources against *H. contortus* and other nematodes species under experimental conditions of infections (G= goats, S= sheep, D= deer; NP= not performed; NS= non significant)

Plant/resource	Botanical family	**Host**	**Nematode parasite**				**Main findings**				**References**
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Temperate legumes											
Ceratonia siliqua	Leguminosae	S	x		*x*		Reduced	NP	Lower *T. colubriformis* total burden. No effect on *H. contortus* burden	Lower fecundity for *T. colubriformis.* No effect on *H. contortus*	Manolaraki et al., 2010
		S	x		x		NS	NP	NS	Lower fecundity for *H. contortus,* it was NP for *T. colubriformis*	Arroyo-Lopez et al., 2014
Hedysarum coronarium	Leguminosae	D		x		*T. axei, Cooperia sp, Oesophagostomum sp*	Trend to be lower	Trend to be lower	Lower abomasal parasite burden. non effect one either small or large intestine species	Fecundity did not differ between species	Hoskin et al., 2000
Lespedeza cuneata	Leguminosae	G	x	x	x		Reduced	Lower percentage of total L3 recovered (especially *H. contortus*)	Lower abomasal (*H. contortus, Te. circumcincta*) and intestinal worms (*T. colubriformis*) counts	NP	Shaik et al., 2006
Lotus pedunculatus	Leguminosae	S		x	x		Reduced	NP	Lower *Te. circumcincta* burden, especially females. *T. colubriformis* was not affected	No inhibited L4's were observed. Fecundity= no effect on *Te. circumcincta* females was observed increased for *T. colubriformis* females	Niezen et al., 1998a
Lotus corniculatus	Leguminosae	D		x		*T. axei, Cooperia sp, Oesophagostomum sp*	Trend to be lower	Trend to be lower	Lower abomasal parasite burden. No effect on neither small or large intestine species	Fecundity did not differ between species	Hoskin et al., 2000
		S	x			*Cooperia curticei*	Reduced	NP	Lower (but NS) *H. contortus* total worm and female burden. No effect on *C. curticei*	NP	Heckendorn et al., 2007

Continuation… Table 3

Plant/resource	Botanical family	Host	Nematode parasite				Main findings				References
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Onobrychis viciifolia	Leguminosae	S	x		x		Reduced	NP	Lower *H. contortus* total burden. NS for *T. colubriformis*	Lower fecundity for *H. contortus,* it was NP for *T. colubriformis*	Arroyo-Lopez et al., 2014
		S	x		x		Lower but NS	NP	NS	NP	Girard et al., 2013
		S	x			*Cooperia curticei*	Reduced	NP	Lower *H. contortus* burden; *C. curticei* counts were reduced but NS	Lower fecundity for *C. curticei.* No effect on *H. contortus* female fecundity	Heckendorn et al., 2006
		S	x			*Cooperia curticei*	Reduced	NP	Lower (but NS) *H. contortus* total and female burden. None effect on *C. curticei*	NP	Heckendorn et al., 2007
		S	x		x		Reduced	NP	Lower *T. colubriformis* burden	Lower fecundity for *H. contortus* and *T. colubriformis*	Manolaraki et al. 2010
		S	x	x	x	*Nematodirus*	Reduced	NP	Lower *Te. circumcincta* and *Nematodirus* burden	NP	Werne et al., 2013a
		S	x	x	x		Reduced	Increasing *H. contortus* L3 proportion	NP	NP	Werne et al., 2013b
Vicia faba	Leguminosae	S	x	x	x		NS	Increasing *H. contortus* L3 proportion	NP	NP	Werne et al., 2013b
Tropical legumes											
Caesalpinia crista	Leguminosae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003
Lysiloma latisiliquum	Leguminosae	G	x		x		NP	NP	Lower total worm burden for both species	Lower establishment for both especies	Brunet et al., 2008

Continuation… Table 3

Plant/resource	Botanical family	Host	Nematode parasite				Main findings				References
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Woody plants											
Corylus avellana	Betulaceae	G	x		x		Reduced	Lower *H. contortus* L3 vs *T. colubriformis*	NS	Lower fecundity for *H. contortus*	Desrues et al ., 2012
		S	x		x		Trends for lower FEC (p < 0.09) after hazelnut skin addition to sainfoin pellets	NP	NS	NP	Girard et al., 2013
Pistacia lentiscus	Anacardiaceae	G		x	x	*C. ovina*	reduced (p< 0.001-0.0001)	NP	NP	NP	Landau et al., 2010
		S	x		x		reduced	NP	NS	Lower fecundity for *H. contortus* and *T. colubriformis*	Manolaraki et al., 2010
Schinopsis spp (QUEBRACHO)	Anacardiaceae	S	x	x	x	*Nematodirus battus*	Reduced only in sheep infected with *T. colubriformis* or *Nematodirus*	NP	Lower *T. colubriformis* and *Nematodirus* burden. Had not effect on abomasal species burden	Lower fecundity of *T. colubriformis* and *Nematodirus* females	Athanasiadou et al., 2001
		G		x	x		Reduced	NP	NS	Lower fecundity for *T. colubriformis* and a trend for *Te. circumcincta*	Paolini et al., 2003
		G		x	x		NP	NP	Lower *T. colubriformis* burden; trend to lower *Te. circumcincta* worm count (p< 0.08).	The fecundity was no affected for both parasites	Paolini et al., 2003
Vernonia anthelmintica	Asteraceae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003
Quercus coccifera	Fagaceae	S	x		x		Reduced	NP	Lower *T. colubriformis* burden. No effect on *H. contortus* burden	Lower fecundity for *T. colubriformis,* trend for *H. contortus* (p< 0.06)	Manolaraki et al. 2010
Melia azedarach	Meliaceae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003
Azadirachta indica	Meliaceae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003

Continuation… Table 3

Plant/resource	Botanical family	Host	Nematode parasite				Main findings				References
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Woody plants											
Fumaria parviflora	Papaveraceae	S	x		x		Reduced	NP	Lower *H. contortus* and *T. colubriformis* burden	No differences in fecundity for both species	Hördegen et al., 2003
Embelia ribes	Primulaceae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003
Salix	Salicaceae	S	x	x	x	*Oesophagostomum, Cooperia, Trichuris, Nematodirus, C. ovina*	Reduced	Decreased in both abomasal species L3. NS for *Trichostrongylus* spp, *C. curticei* or *N. spathiger*	Abomasum: lower *H. contortus* (male and female) and *Te. circumcincta* (female). Small intestine: lower *C. curticei* burden. *Trichostrongylus* and *Nematodirus* were not affected	Lower fecundity in abomasum *Te. circumcincta*, and small intestine *Trichostrongylus* spp. NS for the other species	Mupeyo et al., 2011
Shrubs											
Ananas comosus	Bromeliaceae	S	x		x		NS	NP	NS	No differences in fecundity for both species	Hördegen et al., 2003
Erica umbellata	Ericaceae	G		x	x		Reduced	NP	NS	Reduction in larvae establishment for both species (a trend for *T. colubriformis* $p < 0.09$). Lower fecundity and length for *Te. circumcincta.* No effect on female fertility for *T. colubriformis* but worm length was greater.	Moreno-Gonzalo et al., 2013, 2014
Erica cirenea	Ericaceae	G		x	x						Moreno-Gonzalo et al., 2013, 2014
Calluna vulgaris	Ericaceae	G		x	x						Moreno-Gonzalo et al., 2013, 2014

Table 4: Compared *in vivo* anthelmintic efficacy of tannin-containing resources against *H. contortus* and other GIN species under conditions of natural infections (G= goats, S= sheep; NP= Not performed; NS= non significant)

Plant/resource	Botanical family	Host	**Nematode parasite**				**Main findings**				**References**
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Temperate legumes											
Hedysarum coronarium	Leguminosae	S	x	x	x	*Trichostrongylus* spp, *Nematodirus, Cooperia*	Reduced	NP	Lowest abomasal and intestinal parasite burden. *Trichostrongylus* spp were the most affected	Fecundity or length were not affected	Niezen et al., 1998b
		G		x	x	*T. vitrinus, Trichuris ovis*	NP	NP	No differences between *Te. circumcincta, Trichostrongylus* spp, or *Trichuris* burdens [short term consumption]	NP	Pomroy and Adlington, 2006
Lespedeza cuneata	Leguminosae	G	x	x	x		Reduced	NP	Lower both abomasal species burden. However, *T. colubriformis* was NS	Lower abomasal L4's in the 95% *L. cuneata* diet	Gujja et al., 2013
		G	x	x	x		Reduced	NP	Lower for both abomasal species burden (trend), significantly for *H. contortus* males ($p < 0.05$). The intestinal *T. colubriformis* showed a trend to have lower burden as well lower males ($p < 0.11$)	NP	Mechineni et al., 2014
		G	x	x	x	*Nematodirus*	Reduced	NP	Lower *Te. circumcincta*, *H. contortus* and *T. colubriformis* burdens, but *Nematodirus* was not significant	NP	Min et al., 2005
		G	x	x	x	*T. axei*	Reduced	Decreasing *H. contortus* proportion	Overall reduced, NS burden. Lowest abomasal (*Te. circumcincta*, *H. contortus and T. axei*) than *T. colubriformis* worm counts	NP	Moore et al., 2008
		G	x	x	x		Reduced	Reduced L3 recovered, especially *H. contortus*	Abomasum: lower *H. contortus* burden, *Te. circumcincta* was not affected. Intestine*: T. colubriformis* trend to have lower burden ($p < 0.10$)	Female : male ratio was not affected	Terrill et al., 2007
		G	x	x	x		Reduced	Slight reduction on L3 recovered	Lower abomasal burden, due to *H. contortus* reduction. *Teladorsagia* and *T. colubriformis* were not affected	NP	Terrill et al., 2009
Lotus corniculatus	Leguminosae	S	"abomasum and small intestine spp"				reduced	NP	Lower both abomasal and small intestinal worm burden	Reduced	Marley et al., 2003
		S	x	x	x	*Trichostrongylus* spp, *Nematodirus, Cooperia*	NS	NP	Abomasum: *Trichostrongylus* spp showed the higher burden. Intestine: *Nematodirus* trend to have higher worm counts. No effect on either *Trichostrongylus or Cooperia*	Fecundity or length were not affected. Abomasal *Trichostrongylus* spp that were longer	Niezen et al., 1998b
		S	x	x	x	*Trichostrongylus* spp, *Nematodirus, Cooperia, T. ovis, Chabertia ovina, Oesophagostomum & Trichuris*	NS	Reduced *T. colubriformis* L3	Lower abomasal (*H. contortus*, *Te. circumcincta*) and intestinal (*Nematodirus*, *Cooperia*) worm burden, but higher *T. colubriformis, C. ovina, Oesophagostomum* and *T. ovis*.	Were found higher female worms for abomasal or intestinal especies, except for *Cooperia*	Ramirez-Restrepo et al., 2005

Continuation… Table 4

Plant/resource	Botanical family	Host	**Nematode parasite**				**Main findings**				**References**
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Onobrychis viciifolia	Leguminosae	G	x	x	x		Reduced	NP	Reduced *T. colubriformis* burden (-50%) but NS regard the two other nematodes	Lower fecundity for all abomasal and intestinal species	Paolini et al., 2005
Lotus pedunculatus	Leguminosae	S		x	x		NS	NP	Abomasum: *Trichostrongylus* spp showed the higher burden. Intestine: *Nematodirus* trend to have higher worm counts. No effect on either *Trichostrongylus* or *Cooperia*	Fecundity or length were not affected, except for abomasal *Trichostrongylus* spp that were longer	Niezen et al., 1998a
Trifolium pratense	Leguminosae	S		x		*Trichostrongylus* spp, *Cooperia, Nematodirus*	Reduced	NP	Abomasum: lower *Te. circumcincta* total and male burden. Intestine: trend to have higher *Trichostrongylus* burden (reinfection= lambs treated with AH)	NP	Marley et al., 2005
Trifolium repens	Leguminosae	S		x		*Trichostrongylus* spp, *Cooperia, Nematodirus*	Reduced	NP	Lower *Te. circumcincta* total and male burden. For intestinal species total adult count were not affected, but fewer male were found	NP	Marley et al., 2005
Tropical legumes											
Acacia mearnsii	Leguminosae	S	x		*x*	*Oesophagostomu, Cooperia, Strongyloides papillosus, T. globulosa*	Reduced	NP	Lower *T. colubriformis* and *Cooperia spp* total worms count. Not differences were found to the other nematode species	NP	Cenci et al., 2007
		G	x		x	*Oesophagostomum, Trichuris*	Reduced	NP	No differences related to species, total or sex worms burden	NP	Costa-Júnior et al., 2014
		S,G	x		x	*Oesophagostomum, Cooperia, Strongyloides, Bunostomum*	Slightly reduced in sheep (NS). NS in goats	NS	Sheep: lower *H. contortus* burden. Small intestine: significant increase of worms count, especially T. *colubriformis*	NP	Max, 2010
		S	x		x		Reduced	NP	Lower *H. contortus* burden. No differences for *T. colubriformis*	NP	Minho et al., 2008

Continuation… Table 4

Plant/resource	Botanical family	Host	Nematode parasite				Main findings				References
			H. contortus	*Te. circumcincta*	*T. colubriformis*	Other species	FEC	Larval culture	Adult worms	other	
Woody plants											
Schinopsis spp (QUEBRACHO)	Anacardiaceae	S		x		*T. vitrinus*	NS	NP	NS differences for abomasal or intestinal species	NP	Dawson et al., 2011
Trianthema portulacastrum	Aizoaceae	S	x		x	*Oesophagostumum* & *T. ovis*	Reduced	Lower L3 for the abomasal, small and large intestine species	NP	NP	Hussain et al., 2011
Salix	Salicaceae	S	x	x	x	*Oesophagostomum*, *Cooperia*, *Trichuris*, *Nematodirus*, *C. ovina*	NS	NP	Abomasum: lower *Te. circumcincta* burden. Small intestine: lower burden (*Nematodirus* and *T. colubriformis*). Large intestine: increased proportion of male for *C. ovina* and *T. ovis*	Female: male ratio did not differ for abomasum or small intestine species	Diaz Lira et al., 2008
		S	x	x	x	*Cooperia*, *C. ovina*, *Oesophagostomum*	NS	Reduced L3 proportion for abomasal species, but the opposite for small (*Trichostrongylus* spp, *Cooperia* spp) and large intestine (*C. ovina*, *Oesophagostomum*)	NP	NP	Ramírez-Restrepo et al., 2010
Zanthoxylum rhoifolium	Rutaceae	S	x		x	*Cooperia*, *Oesophagostomum*	NS	NP	NS for *H. contortus*. Reduced intestinal species, especially *Oesophagostomum.*	NS	Peneluc et al., 2009
Shrubs											
Erica spp	Ericaceae	G		x	x	*C. ovina*	Reduced (just in two months/ 5 months studied)	*T. colubriformis* L3 was the most frequent. *C. ovina* was not observed in heather group	NP	NP	Osoro et al., 2007
Calluna vulgaris	Ericaceae	G		x	x	*C. ovina*	Idem	Idem	NP	NP	Osoro et al., 2007
Musa paradisiaca	Musaceae	S	x		x	*Oesophagostumum* & *Trichuris*	Reduced	Lower L3 for the abomasal, small and large intestine species	NP	NP	Hussain et al., 2011

Table 5: Tannin composition in plants or extracts of forage legumes, browse species, fruit bushes and agro-industrial by-products (**PC/PD** = ratio of procyanidins and prodelphinidins; **mDP** = mean Degree of Polymerisation; **CT** = condensed tannins). (*) the meaning for *in vitro* assays are provided in Table 1

Plants	**PC/PD**	**mDP**	**CT** (g/100g DM = %)	**Tannin method**	**Effects on *Haemonchus* (*)**	**References**
Forage Legumes						
Desmodium intortum			√	HCl-ButOH	LMIA; feeding reduced worm burdens, female/male ratio, eggs in uterus of female worms	Debela et al., 2012; Mbugua et al., 2008
Hedysarum coronarium	11/89 to 27/73	3 to 46	3 to 13% DM	Thiolysis; HCl-ButOH	LMIA, EHA	Molan et al., 2004; Tibe et al., 2011 Valderrabano et al., 2010
Lespedeza cuneata (grazed)	3/97	33	13% DM	thiolysis	see SL section	Mechineni et al., 2014
Lespedeza cuneata (pellets)	3/97	86	13	thiolysis	see SL section	Kommuru et al., 2014
Lotus corniculatus	34/66 to 60/40 67/33 to 79/21 73/27 to 50/50	9 to 14	3 to 5% DM		EHA	Acharya et al., 2014; Foo et al., 1982, 1996; Hedqvist et al., 2000; Sivakumaran et al., 2006
Onobrychis viciifolia	34/66 to 5/95	11 to 84	0.6 to 14	Thiolysis; HCl-ButOH	LEIA; FEC; worm numbers; female fecundity	Arroyo-Lopez et al., 2014; Azuhnwi et al., 2013; Quijada et al., 2015; Stringano et al., 2012; Theodoridou et al., 2011
Browse:						
32 different browse species				several species had tannins (PVPP)	LDA, AMIA	Kotze et al., 2009
40 plants (herbs, shrubs, trees)				CT, GT, ET	EHA, LMIA	Acharya et al., 2014
Acacia nilotica (fruit)				epigallocatechin gallates	AMIA, EHA, LDA, development, FEC	Bachaya et al., 2009; Ncube and Mpofu 1994
Acacia nilotica (AN) *leaves*				catechin gallates	AN less effective than AK: FEC, worm number	Kahiya et al., 2003; Self et al., 1986
A. karroo (AK) (leaves)			√	HCl-ButOH	AK more effective than AN: FEC, worm number	Dube and Ndlovu 1995; Dube et al., 2001; Kahiya et al., 2003
Arachis pintoi, Glicidia sepium, Cratylia argentea (leaves)				tannins (PEG)	LMIA, LEIA	von Son-de Fernex et al., 2012
Acacia pennatula, Lysiloma latisiliquum, Piscidia piscipula				vanillin-HCl, tannins (PEG)	LMIA	Calderon-Quintal et al., 2010
Betula spp	59/41	4	5 %		LEIA	Quijada et al., 2015; Ropiak et al. (submitted)
Ceratonia siliqua pods			√	CT dimers & trimers, HT, galloylated sugars; flavan-3-ol gallates	no significant effects on FEC, worm numbers, female fecundity	Arroyo-Lopez et al., 2014; Papagioannopoulos et al., 2004
Ficus infectoria/Psidium guajava leaf meal mixture			√	HCl-ButOH	Worm numbers, FEC	Pathak et al., 2013
Juglans regia	100/0	5	6 %	Thiolysis	LEIA	Quijada et al., 2015; Ropiak et al. (submitted)

Continuation… Table 5

Leucaena leucocephala			3 %	HCl-ButOH	Contradictory reports: a) *in vitro* L_3 toxicity; b) no effect on LMIA	Nguyen et al., 2005; Osborne and McNeill 2001; a) Lopez et al., 2005; b) Calderon-Quintal et al., 2010
Calluna vulgaris, Ericaca cinerea, E. umbellata			√	HCl-ButOH; thiolysis	EHA, LEIA	Betts et al., 1967; Moreno-Gonzalo et al., 2013
Piliostigma thonningii (bark)				HCl-ButOH; vanillin-HCl	L3 lethality; inhibition of glutathione S-transferases	Fakae et al., 2000
Pistacia lentiscus				a) 20% tannins in DM (PEG); b) 5% of gallotannins in DM (galloyl derivatives of glucose, quinic acid and myricetin) c) 3-4% CT (HCl-ButOH)	LEIA; very low FEC	a) Azaizeh et al., 2013; Landau et al., 2010; Manolaraki et al., 2010; b) Rodriguez-Perez et al., 2013 ; Romani et al., 2002 c) Dahmoune et al., 2014; Mancilla-Leyton et al., 2014
Psidium guajava/ Eugenia jambolana				HCl-ButOH	FEC	Singh et al., 2015
Salix sp	5/95 to 96/4	2 to 18	0.2 to 13	Thiolysis; HCl-ButOH	LEIA, worm numbers, FEC	Diaz Lira et al., 2008; Falchero et al., 2011; Klongsiriwet et al., 2015; Mupeyo et al., 2011; Orians et al., 2000; Quijada et al., 2015; Ramírez-Restrepo et al., 2010
Sesbania sesban			√	HCl-ButOH	LMIA	Debela et al., 2012; Heering et al., 1996
Veronia amygdalina			√		worm mortality	Sirama et al., 2015
Fruit bushes						
Ribes nigrum	5/95 to 9/91	3 to 12	10 to 20	Thiolysis	LEIA	Klongsiriwet et al., 2015; Quijada et al., 2015; unpublished data
Ribes rubrum	5/95 to 14/86	5 to 19	-	Thiolysis	LEIA	Klongsiriwet et al., 2015; Quijada et al., 2015

Continuation... Table 5

Agro-industrial byproducts						
Corylus avellana	80/21 to 82/18	5 to 9	-	Thiolysis; CT gallates	LEIA	Del Rio et al., 2011; Quijada et al., 2015
Pinus spp	89/11 to 85/15	2 to 11	10%	Thiolysis; HCl-ButOH	LEIA, FEC, worm numbers	Min et al., 2015a, 2015b; Quijada et al., 2015
Other potential useful plants					**Information on *H. contortus* are missing**	
L. pedunculatus	16/84 to 20/80	12 to 44	7% DM	Thiolysis, phloroglucinolysis	*Ostertagia (Te.) circumcincta* and *Trichostrongylus colubriformis*	Foo et al., 1997 ; Niezen et al., 1998; Sivakumaran et al., 2006
Dorycnium spp	5/95 to 17/83	10 to 127	15 to 20% DM	Thiolysis,	EHA; LDA – *Te. circumcincta*	Molan and Faraj 2010; Sivakumaran et al., 2004
Herbs						
Rumex obtusifolius	100/0	2 to 8	-	Thiolysis; 10 to 29 % galloylation; A-type CT	EHA; LDA – *Te. circumcincta*	Molan and Faraj 2010; Spencer et al., 2007

√ indicates that presence of condensed tannins has been confirmed.

(*) tests showed effects on *Haemonchus contortus* (unless otherwise indicated) sees Table 2 for the *in vitro* assays

Table 6: Browse tannins analysed by thiolysis with benzyl mercaptan. Most results are still unpublished data except the species indicated by * (see Ropiak et al., submitted).

Plants	**Plant parts**	**PC/PD**	**mDP**	**CT** (g/100 g DM)
Amelanchier canadensis	Leaves	100/0	5	4
Arbutus unedo	Leaves	60/40	4	5
Corylus avellana	Leaves	26/74	10	5
Euonymus alatus	Leaves	73/27	8	6
Parrotia persica	Leaves	12/88	27	6
Picea abies	Leaves	85/15	5	4
Pimenta officinalis	Fruits	37/63	14	3
*Pinus spp.**	Buds	62/38	8	5
Platanus x hybrid	Leaves	32/68	13	10
Robinia pseudoacacia	Leaves	7/93	34	14
Salix spp.*	Bark	100/0	5	15
Taxus baccata	Needles	51/49	4	6
*Vaccinium vitis-idaea**	Leaves	100/0	7	17
*Vaccinium myrtillus **	Leaves	100/0	7	12

ETUDE EXPERIMENTALE PERSONNELLE

CHAPITRE 1

CHAPITRE 1 : Etudes *in vitro* des relations structure/activité des tanins condensés sur les nématodes des petits ruminants

ARTICLE 1 : L'activité anthelminthique des tanins condensés contre *Haemonchus contortus* ou *Trichostrongylus colubriformis* parasites des petits ruminants, est influencée par leurs caractéristiques structurelles.

QUIJADA J., FRYGANAS, C., ROPIAK, H.M., RAMSAY, A., MUELLER-HARVEY, I. et HOSTE, H.

2015. *Journal of Agricultural and Food Chemistry,* 63, 6346-6354.

RESUME – ARTICLE 1

Introduction

La consommation des plantes contenant des tanins condensés (TCs) a été associée à des effets néfastes sur les nématodes gastro-intestinaux (GIs) dans de nombreuses études *in vitro* ou *in vivo*. Néanmoins, la forte variation entre résultats anthelminthiques d'études faites avec les mêmes ressources de TCs, mais aussi entre les espèces des nématodes de l'abomasum ou d'intestin, a mené à l'hypothèse que les caractéristiques quantitatives et qualitatives des TC peuvent moduler leur activité contre les vers.

Notre étude a eu pour objectifs : i) d'évaluer les relations entre les structures des TCs et leur activité anthelminthique, en utilisant 36 fractions purifiées des TCs divergeant la taille moléculaire et le rapport prodelphinidols et procyanidols (PD/PC) ou le ratio *trans*/*cis* flavan-3-ols ii) d'évaluer si il existe des variations de réponse selon les espèces de nématodes de l'abomasum et de l'intestin grêle chez les petits ruminants.

Matériel et méthodes

36 fractions purifiées de TCs provenant de 18 ressources (15 plantes), ont été caractérisée quantitativement et qualitativement. A partir de ces 18 ressources, deux types de fractions ont été purifiées, en utilisant une méthode d'élution par acétone/H_20. Ces fractions purifiées de TCs ont été évaluées sur des larves infestantes (L3) obtenue sur des caprins infestés expérimentalement avec une seule l'espèce de l'abomasum (*H. contortus*) ou de l'intestin grêle (*T. colubriformis*). L'activité anthelminthique des TCs a été évaluée en appliquant le test d'inhibition de dégainement larvaire (LEIA) pour chaque espèce de nématode, avec une gamme des concentrations de 37,5 µg/mL à 600 µg/mL à fin de mesurer les cinétiques de dégainement selon la concentration appliquée. Pour chaque fraction (F1 ou F2) la concentration efficace pour obtenir le 50% de l'inhibition du dégainement (CE50) a été calculée.

Des tests non-paramétriques de corrélations de Spearman, ainsi que des analyses multivariées (ACP = Analyse en Composantes Principales) ont été appliquées sur les données de caractéristiques chimiques des fractions de TCs, de même sur les valeurs de CE50 obtenues par les tests de dégainement, toujours sur les différents fractions F1 et F2, mais aussi sur le lot complet des F1+F2.

Résultats et discussion

La caractérisation chimique des 18 ressources, a montré différentes valeurs moyenne de contenu de TC, taille (mDP= « mean degree of polymerization », degré moyen de polymérisation), de PD/PC et *cis/trans* ratio. Globalement, les F2 ont présenté une plus forte concentration de TC, et des ratios PD/PC plus élevés.

Par ailleurs, pour toutes les fractions (F1 et F2) l'activité anthelminthique a suivi une dose-réponse. En général, sur les deux espèces de nématodes, les valeurs calculées des CE50 ont été plus basses pour les F2 que pour les F1. De plus, les CE50 obtenues pour *H. contortus* ont été plus basses que celles pour *T. colubriformis*. Ce résultat suggère une plus forte sensibilité de *H. contortus* par rapport à *T. colubriformis*.

En ce qui concerne l'activité anthelminthique et les caractéristiques chimiques des fractions, pour *H. contortus* une relation a été observée entre les CE50 et les valeurs des mDP (taille) et les ratios PD/PC. Pour *T. colubriformis*, les CE50 ont été corrélées uniquement au fort ratio PD/PC.

En conclusion, une différence a été observée selon l'espèce, *H. contortus* étant la plus sensible. Des modes d'action différents des TCs sont suggérés selon l'espèce parasitaire, étant le ratio PD/PC et la taille (mDP) les caractéristiques chimiques associées à leur activité anthelminthique.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Anthelmintic Activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from Small Ruminants Are Influenced by Structural Features of Condensed Tannins

Jessica Quijada,[†,‡] Christos Fryganas,[§] Honorata M. Ropiak,[§] Aina Ramsay,[§] Irene Mueller-Harvey,[§] and Hervé Hoste*,[†,‡]

[†]INRA, UMR 1225 IHAP, 23 Chemin des Capelles, Toulouse F-31076, France
[‡]Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France
[§]School of Agriculture, Policy and Development, University of Reading, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom

ABSTRACT: Plants containing condensed tannins (CTs) may hold promise as alternatives to synthetic anthelmintic (AH) drugs for controlling gastrointestinal nematodes (GINs). However, the structural features that contribute to the AH activities of CTs remain elusive. This study probed the relationships between CT structures and their AH activities. Eighteen plant resources were selected on the basis of their diverse CT structures. From each plant resource, two CT fractions were isolated and their in vitro AH activities were measured with the larval exsheathment inhibition assay, which was applied to *Haemonchus contortus* and *Trichostrongylus colubriformis*. Calculation of mean EC_{50} values indicated that *H. contortus* was more susceptible than *T. colubriformis* to the different fractions and that the F1 fractions were less efficient than the F2 ones, as indicated by the respective mean values for *H. contortus*, F1 = 136.9 ± 74.1 μg/mL and F2 = 108.1 ± 53.2 μg/mL, and for *T. colubriformis*, F1 = 233 ± 54.3 μg/mL and F2 = 166 ± 39.9 μg/mL. The results showed that the AH activity against *H. contortus* was associated with the monomeric subunits that give rise to prodelphinidins ($P < 0.05$) and with CT polymer size ($P < 0.10$). However, for *T. colubriformis* AH activity was correlated only with prodelphinidins ($P < 0.05$). These results suggest that CTs have different modes of action against different parasite species.

KEYWORDS: *proanthocyanidins, larval exsheathment inhibition assay (LEIA), nematodes, ruminants, structure−activity relationships*

INTRODUCTION

Gastrointestinal nematodes (GINs) represent a major threat for the breeding and production of grazing ruminants. Up to now, their control has been based mainly on the repeated use of synthetic anthelmintic (AH) drugs. However, worm populations in small ruminants have consistently developed resistance against all AH drugs.[1] Therefore, the search for alternative solutions to such drug treatments is now a necessity for a more sustainable control of these parasites.[2] The past two decades have provided evidence that some plants possess natural AH bioactivity, which is based on the presence of condensed tannins (CTs) and flavonoids. Such plants, therefore, represent a promising alternative to chemotherapy, especially when used as nutraceuticals that combine beneficial effects on health and nutrition in small and large ruminants.[3−6]

The involvement of CTs in the observed AH effects against parasitic nematodes has been suggested from several results acquired in vitro using either plant extracts or purified CT fractions[7−10] and from in vivo studies with tannin-containing resources.[11−15]

Differences in AH effects have repeatedly been noted between abomasal and intestinal nematode species of both small ruminant and cattle parasites.[12] These observations have been made in in vitro[9,10,16] and in vivo studies with the same CT resources.[13,15,17,18]

Some authors have suggested that different structural features of CTs are involved in their AH effects, namely, (i) CT size;[7,10,19,20] (ii) the type of flavan-3-ol subunits that give rise to either prodelphinidin (PD) or procyanidin tannins (PC);[8,20−22] or (iii) the stereochemistry of the C-ring in these subunits (i.e., *trans* vs *cis* flavan-3-ols).[19,22] Taken together, these observations led us to hypothesize that there are quantitative and qualitative differences between CTs, which determine their activity against parasitic nematodes. There is thus a need to evaluate the structure−activity relationship between tannins and GINs. A better understanding of these plant compounds is also required for a more rational use of these nutraceutical feeds under farm conditions.

Therefore, the objectives of the current study were (i) to examine the relationship between tannin structures and their anthelmintic activities by using 36 different tannin fractions that span CTs with a wide range of sizes and prodelphinidin/procyanidin and *trans*/*cis* flavan-3-ol ratio and (ii) to evaluate whether responses toward CTs differ between abomasal and intestinal small ruminant nematode species.

MATERIALS AND METHODS

Chemicals. Hydrochloric acid (37%, analytical reagent grade), butan-1-ol, acetic acid glacial (analytical reagent grade), acetone

Received: February 14, 2015
Revised: May 6, 2015
Accepted: June 12, 2015
Published: June 12, 2015

DOI: 10.1021/acs.jafc.5b00831
J. Agric. Food Chem. 2015, 63, 6346−6354

(analytical reagent grade), acetonitrile (HPLC grade), dichloromethane (laboratory reagent grade), hexane (GLC, pesticide residue grade), and methanol (HPLC grade) were obtained from Thermo-Fisher Scientific (Loughborough, UK); benzyl mercaptan (BM) was from Sigma-Aldrich (Poole, UK); phosphate buffered saline (PBS) was from Biomérieux (Marcy l'Etoile, France); Sephadex LH-20 was from GE Healthcare (Little Chalfont, UK); and ultrapure water (MQ H_2O) was from a Milli-Q Plus system (Millipore, Watford, UK).

Preparation of Plant Extracts and Tannin Fractions. Eighteen different plant materials were used: aerial plants of *Onobrychis viciifolia* (OV) were collected on June 7, 2012 (Barham, Kent, UK); *Trifolium repens* flowers were collected from at NIAB (Cambridge, UK; sample TRa) or purchased from Zioła z Kurpi (Jednorożec, Poland; sample TRb); *Lespedeza cuneata* (LC) pellets were from Sims Brothers Seed Co. (Union Springs, AL, USA); *Betulae folium* leaves (*Betula pendula* Roth and/or *Betula pubescens* Ehrh.; BP), *Tilia inflorescentia* flowers (T; a mixture of *Tilia cordata, Tilia platyphyllos,* and *Tilia vulgaris* L), *Salicis cortex* bark (SA) from various *Salix* spp. (including *Salix purpurea* L.; *Salix daphnoides* Vill.; *Salix fragilis* L.), and *Ribes nigrum* leaves (sample RNb) were from Flos (Mokrsko, Poland); *Corylus avellana* (CA) pericarp was from Société Inovfruit (Musidan, France); *Juglandis folium* leaves of *Juglans regia* L. (JR) were from Kawon (Gostyń, Poland); inner bark of *Pinus sylvestris* (PS) was from University of Turku (Turku, Finland); *Salix babylonica* catkins (SB) were collected on May 26, 2012 (Emmer Green, UK); *Salix caprea* (SCL and SCT) leaves and twigs were harvested on June 19, 2012 (Goring-on-Thames, UK); *Ribes nigrum* leaves (sample RNa) and *Ribes rubrum* leaves (RR) were collected on August 13, 2012, from Hildred PYO farm (Goring-on-Thames, UK); *Theobroma cacao* beans (TC) were from Peru (imported by "Detox your world" Inc., Norfolk, UK); and *Vitellaria paradoxa* (VP) meal (i.e., residue of VP nuts after fat extraction) was from AarhusKarlshamm Sweden AB, Sweden. Samples OV and TRa were lyophilized; samples PS, CA, SCL, SCT, RNa, and RR were dried at room temperature for <10 days and then stored at room temperature. The different botanical families[23] of each plant are indicated in Table 2.

Extracts were prepared according to the method of Stringano et al.[24] with a few modifications. Plant samples (50 g; <1 mm sieve) were extracted with 70% acetone/H_2O (500 mL, 7:3, v/v) and filtered under vacuum. Chlorophyll and lipids were removed with dichloromethane (125 mL) by liquid–liquid extraction. The remaining solvents were removed from the aqueous phase on a rotary evaporator at 35 °C. The aqueous extracts were centrifuged for 3 min at 4500 rpm (Jouan CR3i multifunction centrifuge) to remove the remaining chlorophyll, insoluble particles, and some precipitates. Extracts were freeze-dried and stored at −20 °C.

Extracts were purified on Sephadex LH-20 chromatographic columns to remove impurities (mainly sugars and small phenolics) with water. Elution with acetone/H_2O (3:7, v/v) yielded fraction 1 CTs (F1); a second elution with acetone/H_2O (1:1, v/v) yielded fraction 2 CTs (F2). In total 36 (18 F1 and 18 F2) fractions were tested using *H. contortus* and *T. colubriformis* infective third-stage larvae (L3).

Tannin Analysis by Thiolytic Degradation and HPLC. The purified CT fractions were subjected to thiolytic degradation as described by Gea et al.[25] with some changes for the analysis of CT contents (% CT) and features (size in terms of mean degree of polymerization, mDP; percentage of prodelphinidins and procyanidins within CTs, % PD and % PC; and percentage of *trans* vs *cis* flavanols, % *trans*, and % *cis*). Freeze-dried samples (4 mg) were weighed into 10 mL glass tubes, and methanol (1.5 mL) was added, followed by acidified methanol (0.5 mL of 3.3% HCl/in MeOH), benzyl mercaptan (50 μL), and a magnetic stirrer. The tube was capped and heated at 40 °C for 1 h in a water bath. Water (2.5 mL) was added to stop the reaction, and the internal standard (0.5 mL of taxifolin: 0.05 mg/mL) was added. Samples were analyzed within 48 h by RP-HPLC.[25]

Gastrointestinal Nematodes. The third-stage larvae (L3) were obtained from feces of donor goats, kept indoors and infected monospecifically, with AH susceptible strains of either *H. contortus* or *T. colubriformis*. The facilities hosting the animals and trial performance met French ethical and welfare rules (agreement C 31 555 27 of August 19, 2010).

Coproculture were maintained for 12 days at 23 °C to obtain the third-stage larvae. Larvae were then recovered from feces using the Baerman technique and stored at 4 °C in a horizontally vented cap flask at a concentration of 1000−1500 L3/mL. Prior to use, the larvae were checked to ensure that at least 90% of them were mobile and ensheathed.

Larval Exsheathment Inhibition Assay (LEIA). The larval exsheathment inhibition assay was performed as described by Bahuaud et al.[26] to compare the inhibitory effects of the various tannin fractions (F1 and F2) on the exsheathment process of *H. contortus* and *T. colubriformis*. For both nematode species a batch of 2-month-old larvae was used to perform the in vitro assays.

Briefly, 1000 ensheathed L3 larvae (*H. contortus* or *T. colubriformis*) were first incubated for 3 h at 20 °C with one of the fractions at serial dilutions from 600, 300, 150, 75, to 37.5 μg/mL in PBS (0.1 M phosphate, 0.05 M NaCl, pH 7.2). In addition to all of the tested fractions, negative controls (L3 in PBS) were run in parallel. After incubation, the larvae were washed and centrifuged, three times in PBS, and then submitted to the artificial exsheathment process by contact with a solution containing sodium hypochlorite (2% w/v) and sodium chloride (16.5% w/v), which had been diluted 1 to 350 in PBS. The exsheathment kinetics were measured under a microscope at ×200 magnification by identifying the proportion of exsheathed larvae. Regular examination was performed at 0, 20, 40, and 60 min after contact with the exsheathment solution. The exsheathment percentage was calculated according to the formula (number of exsheathed larvae) × 100/(number of exsheathed larvae + ensheathed larvae). For each fraction, four replicates were run per concentration and observation time to examine the exsheathment kinetics.

Statistical Analyses of the Results. The effective concentration that causes 50% exsheathment inhibition (EC_{50}) for each tannin fraction was calculated at 60 min (using the software Probit Polo Plus). First, a nonparametric rank correlation of Spearman was calculated using a 2 by 2 correlation to evaluate the relationship between the structural parameters characterizing the tannin fractions and also the relationship between the in vitro AH activity (EC_{50} of each fraction) and quantitative (% CT) and qualitative parameters (mDP, % PD and *trans*) of the respective F1, F2, and combined F1 and F2 (F1+F2) fractions. Significant values ($P < 0.05$) and (close to significance) values ($P < 0.10$) are reported.

Then multivariate analyses, principal component analyses (PCA), were performed separately for each nematode species on the basis of the combined data of F1+F2 to obtain an overall synthesis of the relationships between the effects on larval exsheathment and the main CT features. The five variables composing the column of the two PCA matrices included quantitative (% CT) and qualitative parameters (mDP, % PD and % *trans* values) plus the EC_{50} per species. The 36 rows of the matrix corresponded to the F1 and F2 data of the 18 plant samples. All statistical analyses were performed using Systat 9 software (SPSS Ltd.).

■ RESULTS

Tannin Analysis and Relationships between Structural Parameters. The parameters that characterized the 18 CT samples are provided in Table 1. The average % CT, mean degree of polymerization (mDP), and % prodelphinidins (PD) values were higher in the F2 compared with the F1 fraction, whereas the mean % *trans* values were lower for F2. The Spearman correlation coefficients were positive and significant between the F1 and F2 fractions for mDP ($r = 0.583$, $P < 0.05$, df = 16), % PD ($r = 0.975$, $P < 0.01$, df = 16), and % *trans* ($r = 0.728$, $P < 0.05$, df = 16), which is due to the fact that these 15 plant species produce different CT types. There was no correlation for the % CT in both fractions ($r = 0.082$, NS, df = 16).

When the Spearman correlation test was applied to the combined F1+F2 data ($n = 36$ samples), there were positive

Table 1. Chemical Characterization of Two Tannin Fractions from 18 Plant Resources (F1 and F2 Fractions; % PC = 100 − % PD; % *cis* = 100 − % *trans*)

scientific name	family[23]	common name/sample	% CT ± SD		mDP ± SD		% PD ± SD		% *trans* ± SD	
			F1	F2	F1	F2	F1	F2	F1	F2
Onobrychis viciifolia	Leguminosae	sainfoin/whole plant	37.2 ± 4.5	100 ± 4.1	2.8 ± 0.1	8.7 ± 0.0	72.0 ± 0.3	64.9 ± 0.1	33.3 ± 0.2	20.9 ± 0.3
Trifolium repens[a]	Leguminosae	white clover/flower	11.7 ± 0.4	100 ± 2.4	1.8 ± 0.0	8.6 ± 0.0	98.3 ± 0.3	98.7 ± 0.0	82.2 ± 0.1	41.1 ± 0.6
Trifolium repens[b]	Leguminosae	white clover/flower	13.4 ± 0.4	82.4 ± 2.0	3.1 ± 0.1	12.7 ± 0.0	98.1 ± 0.1	98.8 ± 0.0	74.2 ± 0.2	38.2 ± 0.0
Lespedeza cuneata	Leguminosae	sericea lespedeza/pellets	42.1 ± 0.2	82.6 ± 1.4	5.0 ± 0.0	11.3 ± 0.3	92.4 ± 0.1	92.3 ± 0.0	34.7 ± 0.0	24.8 ± 0.2
Betula spp.	Betulaceae	birch/leaf	12.9 ± 0.3	63.6 ± 2.5	2.2 ± 0.0	8.3 ± 0.1	44.7 ± 0.1	58.9 ± 0.1	59.3 ± 0.1	29.3 ± 0.1
Corylus avellana	Corylaceae	hazelnut/pericarp	49.2 ± 1.1	67.5 ± 0.6	4.6 ± 01	9.2 ± 0.1	18.3 ± 0.9	20.9 ± 0.8	59.0 ± 0.11	52.2 ± 0.35
Juglans regia L.	Juglandaceae	walnut/leaf	21.8 ± 1.4	69.0 ± 1.7	2.9 ± 0.0	12.3 ± 0.1	9.3 ± 0.4	30.9 ± 0.0	56.1 ± 0.0	23.7 ± 0.0
Pinus sylvestris L.	Pinaceae	pine/inner bark	54.0 ± 2.0	79.0 ± 2.4	2.3 ± 0.0	6.6 ± 0.2	15.1 ± 0.6	11.2 ± 1.7	51.9 ± 0.7	21.9 ± 1.8
Tilia L.	Tiliaceae	lime tree/flower	47.5 ± 2.8	91.7 ± 3.8	2.0 ± 0.0	7.9 ± 0.1	1.1 ± 0.0	0.9 ± 0.1	16.4 ± 0.0	4.4 ± 0.1
Salix spp	Salicaceae	white willlow/bark	23.1 ± 1.7	83.3 ± 0.6	2.0 ± 0.1	9.9 ± 0.0	0.0 ± 0.0	6.0 ± 0.0	63.0 ± 0.2	21.9 ± 0.0
Salix babylonica	Salicaceae	weeping willow/catkins	40.2 ± 2.8	97.4 ± 2.2	2.9 ± 0.0	8.0 ± 0.3	24.6 ± 0.1	33.0 ± 1.7	44.5 ± 0.2	42.3 ± 1.2
Salix caprea	Salicaceae	goat willow/leaf	51.5 ± 0.1	83.8 ± 1.8	2.1 ± 0.1	5.3 ± 0.1	5.8 ± 0.3	4.8 ± 0.6	93.2 ± 0.3	95.8 ± 0.2
Salix caprea	Salicaceae	goat willow/twigs	72.0 ± 1.1	93.2 ± 11	2.1 ± 0.0	5.3 ± 0.1	15.6 ± 0.9	21.3 ± 0.71	59.4 ± 0.1	37.2 ± 0.4
Ribes nigrum[a]	Grossilariaceae	black currant/leaf	59.8 ± 1.3	100.0 ± 1.7	2.5 ± 0.0	6.5 ± 0.1	93.7 ± 0.07	94.5 ± 0.11	87.2 ± 0.1	93.0 ± 0.1
Ribes nigrum[b]	Grossilariaceae	black currant/leaf	55.5 ± 3.2	77.1 ± 3.9	3.8 ± 0.0	11.8 ± 0.1	94.0 ± 0.0	95.3 ± 0.0	91.5 ± 0.1	81.2 ± 0.1
Ribes rubrum	Grossilariaceae	red currant/leaf	57.7 ± 9.1	68.2 ± 1.1	4.9 ± 0.0	10.0 ± 0.1	85.8 ± 0.4	90.4 ± 0.1	55.7 ± 1.1	35.6 ± 0.9
Theobroma cacao	Malvaceae	cocoa/seed	58.5 ± 2.9	75.5 ± 8.1	2.3 ± 0.0	5.4 ± 0.1	0.0 ± 0.2	0.0 ± 0.0	8.7 ± 0.2	3.7 ± 0.1
Vitellaria paradoxa	Sapotaceae	shea/meal	33.0 ± 0.6	44.9 ± 0.8	2.2 ± 0.1	4.1 ± 0.1	76.3 ± 0.1	72.5 ± 0.1	41.4 ± 0.3	40.2 ± 0.1
		mean value	40.2 ± 9.2	81.1 ± 7.4	2.8 ± 0.5	8.4 ± 1.3	44.6 ± 20.2	49.7 ± 19.4	56.2 ± 12.4	39.3 ± 13.3

[a] Sample a. [b] Sample b.

DOI: 10.1021/acs.jafc.5b00831
J. Agric. Food Chem. 2015, 63, 6346−6354

Table 2. EC_{50} Values by Parasite and by Fraction (F1 or F2) from Each Tannin-Containing Resource Tested

plant	abbreviation	family[26]	*H. contortus* EC_{50} (95% CI) (μg/mL)		*T. colubriformis* EC_{50} (95% CI) (μg/mL)	
			F1	F2	F1	F2
Onobrychis viciifolia	OVF1/OV2	Leguminosae	62.7 (49.9–76.5)	212 (182–250)	203 (131–322)	147 (99–230)
Trifolium repens (a)	TRaF1/TRaF2	Leguminosae	287 (249–328)	177 (131–239)	110 (82.1–145)	152 (109–210)
Trifolium repens (b)	TRbF1/TRbF2	Leguminosae	37.5 < (0.7–74.4)[a]	37.5 < (0.08–42.4)[a]	132 (92.3–186)	110 (63.2–166)
Lespedeza cuneata	LCF1/LCF2	Leguminosae	78.2 (28.1–157)	37.5 <(2.5–55.3)[a]	198 (108–366)	94.9 (50.5–140)
Corylus avellana	CAF1/C1F2	Corylaceae	166 (82.5–441)	143 (104–170)	351 (287–441)	329 (209–671)
Juglans regia L.	JRF1/JRF2	Juglandaceae	94.7 (65.5–115)	70.6 (46.9–106)	258 (130–386)	243 (169–384)
Betula spp.	BPF1/BPF2	Betulaceae	62.8 (58.6–82)	62.6 (19.0–90.3)	226 (163–335)	125 (86.7–169)
Pinus sylvestris L.	PSF1/PSF2	Pinaceae	236 (192–290)	144 (125–167)	184 (121–305)	135.9 (112–163)
Tilia L. spp.	TF1/TF2	Tiliaceae	113 (82–157)	88.7 (66.1–107)	459 (353–660)	297 (258–335)
Salix spp.	SAF1/SAF2	Salicaceae	188 (137–241)	138 (117–154)	300 (271–333)	191 (126–294)
Salix babylonica	SBF1/SBF2	Salicaceae	174 (120–206)	128 (69.8–166)	181 (152–214)	108 (83.8–132)
Salix caprea (twigs)	SCTF1/SCTF2	Salicaceae	195 (142–266)	132 (97.6–184)	385 (296–459)	125 (94.9–159)
Salix caprea (leaves)	SCLF1/SCLF2	Salicaceae	196 (86–217)	161 (133–191)	377 (316–435)	316 (243–420)
Ribes nigrum (sample a)	RNaF1/RNaF2	Grossilariaceae	145 (85–259)	157 (124–203)	145 (123–169)	89.5 (70.1–111)
Ribes nigrum (sample b)	RNbF2/RNbF2	Grossilariaceae	48.7 (78.1–158)	59.2 (18.5–111)	315 (212–592)	209 (140–344)
Ribes rubrum	RRF1/RRF2	Grossilariaceae		97.8 (85.4–305)	130 (84.5–199)	124 (99.5–152)
Theobroma cacao	TCF1/TCF2	Malvaceae	208 (168–246)	65.2 (34.1–95.7)	76.1 (24.3–130)	122 (94.8–200)
Vitellaria paradoxa	VP1/VP2	Sapotaceae	37.5 < (0.7–29.1)[a]	37.5 < (0.48–36.5)[a]	169 (115–288)	76.0 (65.7–86.7)
		mean values	136.9 ± 74.1	108.1 ± 53.2	233 ± 54.3	166 ± 39.9

[a] Calculation of the EC_{50} values relying on the Polo Plus software gave the following values for the effects against *H. contortus* for *T. repens* (b), fraction F1 = 33.2 μg/mL and fraction F2 = 14.5 μg/mL; for *L. cuneate*, fraction F2 = 29.4 μg/mL; and for *V. paradoxa*, fraction F1 = 13.6 μg/mL and fraction F2 = 16.5 μg/mL.

correlation coefficients between % CT and mDP values ($r = 0.696$; $P < 0.01$; df = 34). A nonsignificant negative correlation existed between % CT and % *trans* ($r = -0.261$; NS; df = 34) and between % PD and mDP values ($r = 0.270$; NS; df = 34). This absence of a link between % PD and mDP is important because column chromatography of CTs from the same plant material tends to lead to fractions, where % PD and mDP are positively correlated (unpublished observations). Therefore, these F1 and F2 fractions enable the investigation of relationships between CT structures and AH activities. Trends were observed for % PD and % *trans* ($r = 0.300$; $P < 0.08$; df = 34).

Anthelmintic Activity. The different fractions affected the larval exsheathment process in a dose-dependent way. The EC_{50} values for each of the F1 and F2 fractions per plant sample were used to characterize the AH activity and are shown for *H. contortus* and *T. colubriformis* in Table 2. For both parasites, EC_{50} values were generally lower with F2 than with F1 fractions. In addition, overall, EC_{50} values calculated for *H. contortus* were lower than those of *T. colubriformis*, suggesting that *H. contortus* was more susceptible to these fractions. Thus, the calculation of Spearman's correlation coefficients between EC_{50} values, obtained respectively for F1 and F2, showed significant and positive values for both species separately, that is, *H. contortus* ($r = 0.642$; $P < 0.05$; df = 15) and *T. colubriformis* ($r = 0.688$; $P < 0.01$; df = 16). However, there were no correlations between the EC_{50} values of the F1 fractions between *H. contortus* and *T. colubriformis* ($r = -0.056$; NS; df = 15) and also not for the F2 fractions ($r = 0.397$; NS; df = 16). Finally, there were also no correlations between the EC_{50} values of both parasite species with the F1+F2 combined data ($r = 0.164$; NS; df = 33).

Figure 1 shows the EC_{50} score values in rank order for *H. contortus* and *T. colubriformis*, respectively. The 25% of the most effective plants against both GIN species (i.e., lowest EC_{50} values) were *Vitellaria paradoxa*, *Trifolium repens*, *Lespedeza cuneata*, *Ribes nigrum*, *Theobroma cacao*, and *Betula* spp. In addition, *Onobrychis viciifolia* was active against *H. contortus*, and *Ribes rubrum* and *Salix babylonica* were active against *T. colubriformis*.

Table 3 presents the Spearman's correlation coefficients between the EC_{50} values and the various CT parameters for both nematode species in terms of the F1, F2, and the combined (F1+F2) data. For *H. contortus*, there were negative trends between EC_{50} and mDP and % PD of the F1 fraction and between EC_{50} and mDP of the F1+F2 data. The correlation between EC_{50} and % PD was negative and significant for the F1+F2 data. Somewhat surprisingly, a significant positive correlation was noted for EC_{50} values and % CT of the F2 fractions.

In contrast, for *T. colubriformis* there were no correlations with mDP or % CT. Instead, negative correlation coefficients between EC_{50} and % PD were close to significance for F1 ($r = -0.453$; $P < 0.10$; df = 16) and F2 ($r = -0.439$; $P < 0.10$; df = 16) and were significant for the combined (F1+F2) fractions ($r = -0.403$; $P < 0.05$; df = 34).

When PCA was applied separately to either *H. contortus* or *T. colubriformis*, the two main components of axis 1 were mDP and % CT. For axis 2, % PD appeared as the key component. The plane defined by the combination of axes 1 and 2 (Figure 2) represented 67% of the overall variability for *H. contortus* and close to 70% for *T. colubriformis*.

The main objective of the PCA was to analyze the overall combined relationships between the different variables and the effects on exsheathment as assessed by the EC_{50} values (Figure 2). Variables that are positively related are located on the same side of the plane. In contrast, variables that are negatively related are located in diagonally opposed quadrants. Analyses of these planes for both GIN species tend to confirm the 2 by 2 Spearman's correlation results. For *Haemonchus*, the EC_{50} values were in opposition to % PD and mDP values and, to a lesser

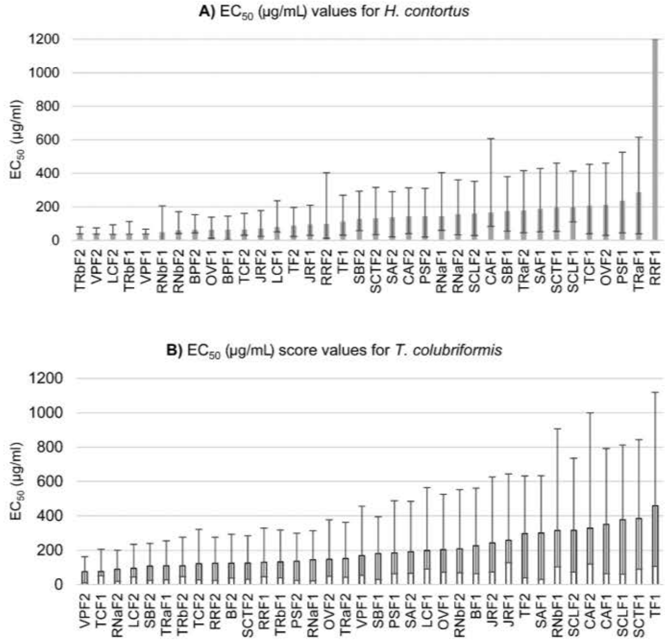


Figure 1. EC_{50} values (and 95% confidence interval) scores for (A) *Haemonchus contortus* and (B) *Trichostrongylus colubriformis* using F1 and F2 fractions from the 18 tannin-containing plant resources.

Table 3. Spearman's Correlation Coefficients for Anthelmintic Activity by Nematode Species According to Tannin Content and Structural Parameters in F1 and/or F2 Fractions

	Haemonchus contortus EC_{50} (µg/mL)						*Trichostrongylus colubriformis* EC_{50} (µg/mL)					
	F1 (df = 15)		F2 (df = 16)		F1+F2 (df = 33)		F1 (df = 16)		F21 (df = 16)		F1+F2 (df = 34)	
variable	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value	*r* value	*P* value
% CT	0.30	0.44	0.61[a]	0.50	0.12	0.29	0.10	0.43	0.01	0.43	−0.22	0.28
mDP	−0.46[b]	0.44	−0.28	0.43	−0.33[b]	0.29	−0.17	0.43	0.19	0.43	−0.26	0.28
% PD	−0.44[b]	0.44	−0.22	0.43	−0.35[a]	0.34	−0.46[b]	0.43	−0.43[b]	0.43	−0.40[a]	0.34
% trans	0.08	0.44	0.12	0.43	0.18	0.29	0.12	0.43	−0.01	0.43	0.24	0.28

[a] $P < 0.05$. [b] $P < 0.10$.

extent, to the % CT. For *Trichostrongylus*, the EC_{50} values were mainly in opposition to % PD.

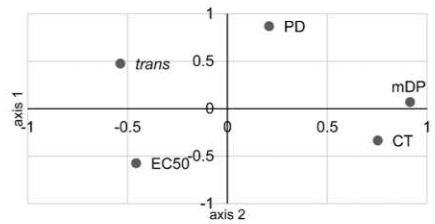
■ DISCUSSION

The study evaluated 36 CT fractions from 18 sources (15 plant species). These plants were chosen because they present a wide range of different CT features in terms of mDP, % PD, and % *trans* values. It was expected that this variation would allow exploring the relationships between CTs and their AH activities. These particular CT parameters have been described previously as being involved in their biological activities.[10,19,20,22,27−29] From these 15 plant species 18 tannin extracts were obtained that yielded two related CT fractions (i.e., F1 and F2 fractions). These 36 samples were used to test the effects of quantitative and qualitative differences between CTs. The range of CT concentrations tested with these fractions was chosen on the basis of previous in vitro data, which had been obtained with plant extracts of known CT concentrations.[16,26,27]

Three in vitro assays are available to explore the interactions between tannins and infective third-stage larvae of gastrointestinal nematodes;[30] these are the larval migration inhibition assay (LMIA), the larval feeding inhibition assay (LFIA), and the LEIA, which has been used in the current study. The LEIA has been widely used to screen the AH activity of either plant extracts,[26,30] tannin fractions,[8,10] or flavan-3-ol monomers.[21,22] The LEIA has proved to be simple and reproducible and, like the LFIA, it also has the advantage that it allows calculation of EC_{50} values, which is rarely the case for the LMIA. Moreover, LEIA has been related to similar in vivo processes.[31] The LEIA was performed with 2-month-old larvae for both nematode species to allow comparison of EC_{50} values obtained with the F1 and F2 fractions of each plant sample and between the two nematodes species.

Overall, CT contents (% CT) were higher in the F2 than in the F1 fractions, and the EC_{50} values for F2 calculated for both nematodes were, in most cases, lower than for F1 fractions. This suggests a role for the % CT in the antiparasitic effect. Similar results were obtained by Williams et al.[20] for the AH

A) PCA values *for H. contortus*



B) PCA for *T. colubriformis*

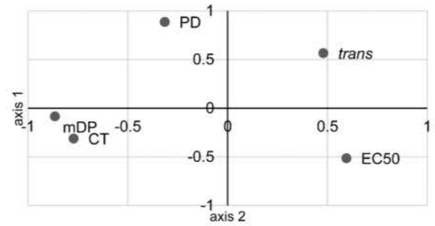


Figure 2. Multivariate principal component analyses (PCA) explained to condensed tannins for each parasite species: (A) *H. contortus*; (B) *T. colubriformis*. For both nematode species, the matrix was composed of 5 variables and 36 lines corresponding to 2 fractions (F1 and F2) of a range of 18 tannin-containing samples. Abbreviations: EC_{50} values based on LEIA (low values reflect high anthelmintic activities), CT (condensed tannins content, units g CT/100 g fraction); mDP (mean degree of polymerization of tannins); PD (% of prodelphinidins) *trans* (% of *trans* flavan-3-ols). The planes represent 67% of the variability for *H. contortus* and 70% for *T. colubriformis*, respectively.

effects against *Ascaris suum* with a subset of these F1 and F2 fractions. Many studies, based on different in vitro tests, have reported a dose-dependent AH effect when using tannin-containing plant extracts. For example, for some legume forages such dose-dependent effects have been described for (i) *O. viciifolia* (sainfoin) with the larval migration inhibition assay (LMIA),[7] LEIA,[31] egg hatch assay (EHA),[28] and larval development inhibition assay (LDIA);[28] and for (ii) *L. pedunculatus* and *L. corniculatus* extracts with the LMIA and LDIA,[27,28] the larval feeding inhibition assay (LFIA), and LEIA.[9] Although, surprisingly, there was a significant positive correlation between CT content and AH activity of the F2 fractions for *H. contortus*, there was no significant correlation when the F1+F2 data were combined. Similarly, Naumann et al.[19] also found no relationship between CT content and the AH activity against *H. contortus* L3 when comparing fractions from three legumes (*Lespedeza stuevei*, *L. cuneata*, and *Arachis glabrata*). Novobilský et al.[10] compared the effects of different CT fractions from *O. viciifolia* on cattle nematodes of either the abomasum (*Ostertagia ostertagi*) or the small intestine (*Cooperia oncophora*). These authors also did not obtain consistent correlations between the CT contents and the in vitro AH activity as measured by LFIA.

This discrepancy in relationship between dose and AH activity obtained with either CT-containing extracts or fractions could perhaps be related to other compounds that are also present in extracts.[7,21] Indeed, Molan et al.[22] also reported deleterious effects of flavan-3-ol monomers against *T. colubriformis* at different life cycle stages, that is, eggs (EHA) and larvae (LDIA, LMIA). The highest AH effect occurred with the epigallocatechin gallate (EGCG) monomer. This observation was confirmed by further studies with green tea fractions that were tested against *Teladorsagia circumcincta* and *T. colubriformis*, where higher EGCG content was linked with a higher AH effect.[8] Similarly, when monomeric subunits of CT were tested in the LEIA on *H. contortus* and *T. colubriformis*,[21] a higher AH activity was observed with (i) the monomeric subunits of PDs (i.e., gallocatechin, epigallocatechin) and (ii) the galloyl derivatives of both PDs and procyanidins.

Besides the possible contribution of CT concentration toward explaining antiparasitic activities, several authors have also suggested that CT structures (or quality) could explain some of the observations.[8−10,19,20,22] For instance, it has been proposed that the biological activity is affected by the hydroxylation at the B-ring in flavan-3-ol monomers and in polymers, where the presence of an additional hydroxyl group (OH) increases the interaction with proteins. This could explain the generally higher activity of PDs compared to PCs. In addition, activity is also increased when galloyl groups are present.[21,32−34]

Results of the 2 by 2 calculations of Spearman's correlation coefficients as well as multivariate analyses (PCA) tended to confirm that the in vitro AH activity in terms of EC_{50} was related to CT structural features for both *H. contortus* and *T. colubriformis*. In addition, our results suggest that different mechanisms appear to be involved for each nematode species. For *H. contortus*, AH activity appeared stronger for CTs with higher PD contents and larger sizes (mDP values), although, as described by Williams et al.,[20] there was no effect of mDP or % PD within F2 fractions on the EC_{50} values. For the F1 fractions, lower EC_{50} values were associated with higher % PD and larger tannins (higher mDP values). Novobilský et al.[10] suggested that mDP was a key factor in the LFIA against L3 of *O. ostertagi* and *C. oncophora* after testing *O. viciifolia* extracts and fractions.

However, Naumann et al.[19] found no clear evidence for CT size and inhibition of *H. contortus* motility. However, only a narrow range of CT sizes was investigated. Conversely to the present data, Manoralaki[35] found that lower mDP values were correlated with higher AH activity when extracts from 40 *O. viciifolia* accessions were tested by LEIA against *H. contortus*. Similarly, Barrau et al.[7] found that a fraction that contained CTs (<2000 Da) plus flavonol glycosides had higher AH effects against *H. contortus* larvae than a fraction that contained only CTs (>2000 Da). At this stage, it is important to note that the complexity of plant extract compositions and difficulties in purifying CTs are likely to account for some of these apparent contradictions. Acetone/water extracts from CT-containing plants consist of CTs plus low molecular weight phenolic compounds (flavones, flavonols, flavonol glycosides, etc.). In addition, CTs usually occur as complex mixtures that contain low to high molecular weight tannins, and the mDP value simply describes the average "tannin size" rather than the distribution profile of all CTs. In fact, we recently discovered that mixtures of CTs and flavonoids had higher AH activities than CTs on their own.[36] Kozan et al.[37] also reported that flavonol glycosides (luteolin-7-*β*-*O*-glucopyranoside and quercetin-3-*O*-*β*-glucopyranoside) from *Vicia pannonica* var. *purpuracens* might also participate in the modulation of bioactivity of the highly AH extract and fractions against trichostrongylid larvae. This emphasizes that the proximity of biochemical structure between flavonol glycosides and CT (which are flavan-3-ols' polymers) could suggest a similar or close mechanism of action for both types of compounds. Taken together, the presence of non-CT compounds (such as flavonoid monomers) could, therefore, explain the apparently contradictory observations by Manoralaki[35] and Barrau et al.[7] The F1 fractions had only half the CT contents of F2 fractions (Table 1). However, the combination of F1+F2 data gave a close to significant correlation of EC_{50} and mDP values (Table 3).

In contrast, for *T. colubriformis*, % PD was consistently (F1, F2, and combined F1+F2) related to AH activity. This agrees with other reports on *T. colubriformis* larvae, which found higher AH in vitro effects of PD-rich compared with PC-rich tannins.[21,22]

Interestingly, there were different susceptibilities between the two parasite species, which suggested that *H. contortus* was more susceptible than *T. colubriformis*. This is indicated by the overall lower EC_{50} values for the abomasal species with both types of CT fractions. Molan et al.[8] also pointed out that the abomasal nematode *T. circumcincta* was more susceptible than *T. colubriformis* to the AH effects of flavan-3-ol monomers and oligomeric CTs in the LMIA. The same conclusion was drawn from in vitro studies that examined extracts from different woody plants (*Rubus fructicosus*, *Quercus robur*, and *Corylus avellana*) against *H. contortus*, *T. circumcincta*, and *T. colubriformis* based on LMIA and LEIA tests.[16] However, other authors found no such differences in the response to quebracho or *O. viciifolia* extracts[11,31] between abomasal or intestinal species. Moreno-Gonzalo et al.[38,39] even found a higher in vitro susceptibility of *T. colubriformis* compared to *H. contortus* and *T. circumcincta* when measuring the AH activity of extracts from different heather species (*Calluna vulgaris*, *Erica cinerea*, and *E. umbellata*). It remains to be seen whether differences in assay conditions could account for some of these contradictory results. Moreover, it will be worth exploring whether there exist species-specific differences in the quality of larval sheath proteins between the abomasal and intestinal species to better understand the mode of actions of polyphenols against the different GIN species.

Although it is difficult to extrapolate from in vitro to in vivo results, our current data provide a screening of CT-containing plants whose AH properties will need to be explored further in controlled in vivo studies to develop their potential for on-farm exploitation. It is also worth noting that the CT fractions from three legumes ranked among the most effective ones (i.e., having the lowest EC_{50} values): *L. cuneata* pellets, *O. viciifolia* plants, and *T. repens* flowers (Figure 1). The past decade has seen an accumulation of in vivo results that confirm the AH effects of *L. cuneata* and *O. viciifolia* against the main GIN species whether offered to small ruminants in the form of freshly grazed pasture[40,41] or as hay,[15,17,42] silages,[42] or pellets.[18]

As far as *T. repens* is concerned, no other data are available because the genus *Trifolium* is usually considered as a tannin-free legume,[43] and consequently the various *Trifolium* species have received little attention for their antiparasitic potential. However, Carlsen and Fomsgaard[44] provided an extensive review of the secondary metabolites in *T. repens* and pointed out the high CT content in flowers. The current study found that CTs from *T. repens* flowers had a strong AH effect and confirmed the dose-dependent inhibition effects of *T. repens* tannins observed for *C. oncophora* in the LFIA.[45]

The CT fractions of *V. paradoxa* were also ranked as highly effective against both nematode species and suggested that some agro-industrial byproducts could be of interest for their antiparasitic properties. It is worth noting that AH effects on *H. contortus* and *T. colubriformis* were recently also described not only for cocoa seed but also for husk extracts using the EHA.[46]

In conclusion, our results showed that structural features of condensed tannins are key factors that affect the anthelmintic effects against gastrointestinal nematodes of ruminants. In addition, there were differences in the susceptibilities of the abomasal as the intestinal nematode species. These differences have been described previously in the literature and could be related to the fact that the nematode sheath proteins differ in these parasite species. This could perhaps affect their interactions with the tannins. It is worth also emphasizing that the current results have been acquired on infective larvae and that other assays that target other parasitic stages might have different outcomes. Further studies will be needed to explore these interactions at the molecular level.

■ AUTHOR INFORMATION

Corresponding Author

*(H.H.) Phone: +(33) 05-61-19-38-75. Fax: +(33) 05-61-19-32-43. E-mail: h.hoste@envt.fr.

Funding

The financial support of the European Commission through the PITN-GA-2011-289377, "LegumePlus" project, is sincerely acknowledged.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank Alison and Dave Prudence for the *S. caprea* leaves and twigs, Hildred's PYO Farm for the *R. nigrum* and *R. rubrum* leaves, and P. Davy for the *O. viciifolia* plants. The *V. paradoxa* meal sample was kindly provided by AarhusKarlshamm Sweden AB, Sweden. We also deeply appreciate all of LegumePlus' fellows for help in collecting plant samples.

■ ABBREVIATIONS USED

GINs, gastrointestinal nematodes; CT, condensed tannins; AH, anthelmintic; mDP, mean degree of polymerization; PD, prodelphinidins; PC, procyanidins; PBS, phosphate buffered saline; LEIA, larval exsheathment inhibition assay; L3, infective stage nematode larvae; EC_{50}, effective concentration for 50% inhibition of larvae's exsheathment; LDIA, larval development inhibition assay; LFIA, larval feeding inhibition assay; EHA, egg hatch assay; LMIA, larval migration inhibition assay

■ REFERENCES

(1) Jackson, F.; Varady, M.; Bartley, D. J. Managing anthelmintic resistance in goats – can we learn lessons from sheep? *Small Ruminant Res.* **2012**, *103* (1), 3−9.

(2) Hoste, H.; Torres-Acosta, J. F. J. Non chemical control of helminths in ruminants: adapting solutions for changing worms in a changing world. *Vet. Parasitol.* **2011**, *180*, 144−154.

(3) Andlauer, W.; Fürst, P. Nutraceuticals: a piece of history, present status and outlook. *Food Res. Int.* **2002**, *35*, 171−176.

(4) Athanasiadou, S.; Houdijk, J.; Kyriazakis, I. Exploiting synergisms and interactions in the nutritional approaches to parasite control in sheep production systems. *Small Ruminant Res.* **2008**, *76*, 2−11.

(5) Hoste, H.; Martinez-Ortiz-de-Montellano, C.; Manolaraki, F.; Brunet, S.; Ojeda-Robertos, N.; Fourquaux, I.; Torres-Acosta, J. F. J.; Sandoval-Castro, C. A. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infection. *Vet. Parasitol.* **2012**, *186*, 18−27.

(6) Terrill, T. H.; Miller, J. E.; Burke, J. M.; Mosjidis, J. A.; Kaplan, R. M. Experiences with integrated concepts for the control of *Haemonchus contortus* in sheep and goats in the United States. *Vet. Parasitol.* **2012**, *186*, 28−37.

(7) Barrau, E.; Fabre, N.; Fouraste, I.; Hoste, H. Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia* Scop.) on the *in vitro*

larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. *Parasitology* **2005**, *131*, 531−538.

(8) Molan, A. L.; Sivakumaran, S.; Spencer, P. A.; Meagher, L. P. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis in vitro*. *Res. Vet. Sci.* **2004**, *77*, 239−243.

(9) Novobilský, A.; Mueller-Harvey, I.; Thamsborg, S. M. Condensed tannins act against cattle nematodes. *Vet. Parasitol.* **2011**, *182*, 213−220.

(10) Novobilský, A.; Stringano, E.; Hayot Carbonero, C.; Smith, L. M. J.; Enemark, H. L.; Mueller-Harvey, I.; Thamsborg, S. M. *In vitro* effect of extracts and purified tannins of sainfoin (*Onobrychis viciifolia*) against two cattle nematodes. *Vet. Parasitol.* **2013**, *196*, 532−537.

(11) Athanasiadou, S.; Kyriazakis, I.; Jackson, F.; Coop, R. L. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.* **2001**, *99*, 205−219.

(12) Hoste, H.; Jackson, F.; Athanasiadou, S.; Thamsborg, S. M.; Hoskin, S. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol.* **2006**, *22*, 253−261.

(13) Joshi, B. R.; Kommuru, D. S.; Terrill, T. H.; Mosjidis, J. A.; Burke, J. M.; Shakya, K. P.; Miller, J. E. Effect of feeding sericea lespedeza leaf meal in goats experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.* **2011**, *178*, 192−197.

(14) Max, R. A. Effect of repeated wattle tannin drenches on worm burdens, faecal egg counts and egg hatchability during naturally acquired nematode infection in sheep and goats. *Vet. Parasitol.* **2010**, *169*, 138−143.

(15) Terrill, T. H.; Dykes, G. S.; Shaik, S. A.; Miller, J. E.; Kouakou, B.; Kannan, G.; Burke, J. M.; Mosjidis, J. A. Efficacy of sericea lespedeza hay as a natural dewormer in goats: dose titration study. *Vet. Parasitol.* **2009**, *163*, 52−56.

(16) Paolini, V.; Fouraste, I.; Hoste, H. *In vitro* effects of three woody plant and sainfoin on third-stage larvae and adult worms of three gastrointestinal nematodes. *Parasitology* **2004**, *129*, 69−77.

(17) Paolini, V.; De La Farge, F.; Prevot, F.; Dorchies, Ph.; Hoste, H. Effects of the repeated distribution of sainfoin hay on the resistant and the resilience of goats naturally infected with gastrointestinal nematodes. *Vet. Parasitol.* **2005**, *127*, 277−283.

(18) Kommuru, D. S.; Barker, T.; Desai, S.; Burke, J. M.; Ramsay, A.; Mueller-Harvey, I.; Miller, J. E.; Mosjidis, J. A.; Kamisetti, N.; Terrill, T. H. Use of pelleted sericea lespedeza (*Lespedeza cuneata*) for natural control of coccidian and gastrointestinal nematodes in weaned goats. *Vet. Parasitol.* **2014**, *204*, 191−198.

(19) Naumann, H. D.; Armstrong, S. A.; Lambert, B. D.; Muir, J. P.; Tedeshi, L. O.; Kothmann, M. M. Effect of molecular weight and concentration of legume condensed tannins on *in vitro* larval migration inhibition of *Haemonchus contortus*. *Vet. Parasitol.* **2014**, *199*, 93−98.

(20) Williams, A. R.; Fryganas, C.; Ramsay, A.; Mueller-Harvey, I.; Thamsborg, S. T. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One* **2014**, *9* (5), No. e97053, DOI: 10.1371/journal.pone.0097053.

(21) Brunet, S.; Hoste, H. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. *J. Agric. Food Chem.* **2006**, *54*, 7481−7487.

(22) Molan, A. L.; Meagher, L. P.; Spencer, P. A.; Sivakumaran, S. Effect of flavan-3-ols *in vitro* hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis*. *Int. J. Parasitol.* **2003**, *33*, 1691−1698.

(23) Blamey, M.; Grey-Wilson, C. *La Flore d'Europe Occidentale*; Flammarion: Paris, France, 2003.

(24) Stringano, E.; Gea, A.; Salminen, J.-P.; Mueller-Harvey, I. Simple solution for a complex problem: proanthocyanidins, galloyl glucoses and ellagitannins fit on a single calibration curve in high-performance-gel permeation chromatography. *J. Chromatogr. A* **2011**, *1218*, 7804−7812.

(25) Gea, A.; Stringano, E.; Brown, R. H.; Mueller-Harvey, I. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. *J. Agric. Food Chem.* **2011**, *59*, 495−503.

(26) Bahuaud, D.; Martinez-Ortiz De Montellano, C.; Chauveau, S.; Prevot, F.; Torres-Acosta, F.; Fouraste, I.; Hoste, H. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology* **2006**, *132*, 545−554.

(27) Molan, A. L.; Alexander, R. A.; Brookes, I. M.; McNabb, W. C. Effect of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes *in vitro*. *Proc. N. Z. Soc. Anim. Prod.* **2000**, *60*, 21−25.

(28) Molan, A. L.; Waghorn, G. C.; McNabb, W. C. The impact of condensed tannins on egg hatching and larval development of *Trichostrongylus colubriformis*. *Vet. Rec.* **2002**, *150*, 65−69.

(29) Mueller-Harvey, I. Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.* **2006**, *86*, 2010−2037.

(30) Jackson, F.; Hoste, H. *In vitro* methods for the primary screening of plant products for direct activity against ruminant gastrointestinal nematodes. In *In Vitro Screening of Plant Resources for Extra Nutritional Attributes in Ruminants: Nuclear and Related Methodologies*; Vercoe, P. E., Makkar, H. P. S., Schlink, A. C., Eds.; FAO/IAEA Springer: Berlin, Germany, 2010; pp 24−45.

(31) Brunet, S.; Aufrere, J.; El Babili, F.; Fouraste, I.; Hoste, H. 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* **2007**, *134*, 1253−1262.

(32) Brunet, S.; Jackson, F.; Hoste, H. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomer of condensed tannins on the association of abomasal nematode larvae with fundic explants. *Int. J. Parasitol.* **2008**, *38*, 783−790.

(33) Li, C.; Leverence, R.; Trombley, J. D.; Xu, S.; Yang, J.; Tian, Y.; Reed, J. D.; Hagerman, A. E. High molecular weight persimmon (*Diospyros kaki* L.) proanthocyanidin, a highly galloylated, A-linked tannin with an unusual flavonol terminal unit, myricetin. *J. Agric. Food Chem.* **2010**, *58*, 9033−9042.

(34) Okuda, T.; Ito, H. Tannins of constant structure in medicinal and food plants − hydrolysable tannins and polyphenols related to tannins. *Molecules* **2011**, *16*, 2191−2217.

(35) Manolaraki, F. *Propriétés anthelminthiques du sainfoin (Onobrychis viciifolia): analyse des facteurs de variations et du rôle des composés phénoliques impliqués.* Ph.D. Dissertation, INP, Université de Toulouse, Toulouse, France, 2011.

(36) Klongsiriwet, C.; Quijada, J.; Williams, A. R.; Hoste, H.; Williamson, E.; Mueller-Harvey, I. 2014. Synergistic effects between condensed tannins and luteolin on *in vitro* larval exsheathment inhibition of *Haemonchus contortus*. In *Proceedings of XXVIIth International Conference on Polyphenols (ICP2014)*, Nagoya, Japan, Sept 2−6, 2014; ISBN 978-4-9907847-0-6), pp 601−602.

(37) Kozan, E.; Anul, S. A.; Tatli, I. I. *In vitro* anthelmintic effect of *Vicia pannonica* var. *purpurascens* on trichostrongylosis in sheep. *Exp. Parasitol.* **2013**, *134*, 299−303.

(38) Moreno-Gonzalo, J.; Manolaraki, F.; Frutos, P.; Hervas, G.; Celaya, R.; Osoro, K.; Ortega-Mora, L. M.; Hoste, H.; Ferre, I. *In vitro* effect of heather (Ericaceae) extracts on different development stages of *Teladorsagia circumcincta* and *Haemonchus contortus*. *Vet. Parasitol.* **2013**, *197*, 235−243.

(39) Moreno-Gonzalo, J.; Manolaraki, F.; Frutos, P.; Hervas, G.; Celaya, R.; Osoro, K.; Ortega-Mora, L. M.; Hoste, H.; Ferre, I. *In vitro* effect of heather extracts on *Trichostrongylus colubriformis* eggs, larvae and adults. *Vet. Parasitol.* **2013**, *197*, 586−594.

(40) Athanasiadou, S.; Tzamaloukas, O.; Kyriazakis, I.; Jackson, F.; Coop, R. L. Testing for direct anthelmintic effects of bioactive forages against *Trichostrongylus colubriformis* in grazing sheep. *Vet. Parasitol.* **2005**, *28*, 233−243.

(41) Mechineni, A.; Kommuru, D. S.; Gujja, S.; Mosjidis, J. A.; Miller, J. E.; Burke, J. M.; Ramsay, A.; Mueller-Harvey, I.; Kannan, G.; Lee, J. H.; Kouakou, B.; Terrill, T. H. Effect of fall-grazed sericea lespedeza (*Lespedeza cuneata*) on gastrointestinal nematode infections of growing goats. *Vet. Parasitol.* **2014**, *29*, 221−228.

(42) Heckendorn, F.; Häring, D. A.; Maurer, V.; Zinsstag, J.; Langhans, W.; Hertzberg, H. Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* and *Cooperia curticei* in lambs. *Vet. Parasitol.* **2006**, *142*, 293−300.

(43) Barry, T. N. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. *Br. J. Nutr.* **1985**, *54*, 217−221.

(44) Carlsen, S.; Fomsgaard, I. S. Biologically active secondary metabolites in white clover (*Trifolium repens* L.) − a review focusing on contents in the plant, plant−pest interactions and transformation. *Chemoecology* **2008**, *18*, 129−170.

(45) Desrues, O.; Enemark, H. L.; Mueller-Harvey, I.; Fryganas, C.; Thamsborg, S. M. Preliminary analysis of the relationship between structure and anthelmintic activity of condensed tannins in cattle nematodes. In *Proceedings 7th Novel Approaches to the Control of Helminths of Livestock*; Toulouse, France, March 25−28; 2013; p 46.

(46) Vargas-Magaña, J. J.; Torres-Acosta, J. F. J.; Aguilar-Caballero, A. J.; Sandoval-Castro, C. A.; Hoste, H.; Chan-Pérez, J. I. Anthelmintic activity of acetone−water extracts against *Haemonchus contortus* eggs: Interactions between tannins and other plant secondary compounds. *Vet. Parasitol.* **2014**, *206*, 322−327.

CHAPITRE 2

CHAPITRE 2 : Etudes *in vitro* des relations structure/activité des tanins condensés et interaction avec d'autres flavonoïdes sur les nématodes chez les petits ruminants

ARTICLE 2: L'inhibition synergique du dégainement d'*Haemonchus contortus* par des monomères flavonoïdes et tanins condensés.

KLONGSIRIWET, C., **QUIJADA J.,** WILLIAMS, A., MUELLER-HARVEY, I., WILLIAMSON, E. et HOSTE, H.

2015. *International Journal for Parasitology: Drugs and Drug Resistance* 5, 127-134.

RESUME – ARTICLE 2

Introduction

Les nématodes gastro-intestinaux (NGIs) chez les petits ruminants, restent un des principaux problèmes pathologiques pour ces espèces, car ils affectent la santé le bien-être animale et la production des animaux. De plus, des résistances aux anthelminthiques de synthèse sont de plus en plus souvent rapportées. Les plantes contenant des métabolites secondaires tels les tanins condensés (TCs) apparaissent désormais comme une méthode de contrôle alternative pour les NGIs. Les TCs sont des oligomères ou des polymères de flavan-3-ols, surtout des types procyanidols (PC) ou prodelphinidols (PD). Les TCs coexistent avec d'autres flavonoïdes dans les plantes, et il est primordial évaluer leurs puissances combinées.

Dans cette perspective, cette étude a eu comme objectifs: i) évaluer si des interactions (synergies ou antagonisme) existent entre des TCs et autres monomères flavonoïdes en se référant au test in vitro de dégainement d'*H. contortus*, et ii) si ces interactions dépendent des types de TCs.

Matériel et méthodes

8 fractions purifiées des TCs (F1, F2) ont été obtenus à partir de 4 ressources de plantes contenant de TCs (*Salix caprae, Ribes nigrum, Ribes rubrum* et *Tilia* spp), sélectionnées en raison de la diversité de caractéristiques (mDP, ratios PD/PC ; *cis /trans*) des TCs présents. Les 2 types de fractions (F1 et F2) ont été obtenues à partir des extraits par élution sur colonne Sephadex LH-20, en utilisant des solutions d'acétone/H_2O (3:7, v/v) pour obtenir les F1, et (1:1, v/v) pour les F2. Toutes ces fractions ont été caractérisées par la methode de Thiolyses au benzyle-mercaptan. L'activité AH des fractions a été évaluée seules ou en combinaison avec deux flavonoïdes. Deux souches d'*H. contortus* susceptibles aux anthelminthiques de synthèse ont été utilisés : Juan (provenant des ovins) et INRA (provenant de caprins).

Les tests d'inhibition de dégainement (« LEIA ») ont été réalisés d'abord avec toutes les fractions seules, les monomères seuls et en combinant fractions et monomères, sur des L3 de la souche Juan, puis seules les F2 (les fractions les plus efficaces) ont été appliqués sur des L3 de la souche INRA.

La concentration efficace pour obtenir une inhibition de 50% de dégainement des larves (CE50) a été calculée pour chaque essai. L'effet inhibiteur additionnel a été prédit en utilisant la définition de Bliss pour comparaison avec les résultats réels.

Résultats et discussion

Les analyses des TCs des fractions ont montré des différences structurelles entre elle. Ainsi, en relation avec le type de TC contenu par ressource, *S. caprae* et *Tilia* spp présentaient surtout des PC, tandis que *R. nigrum* et *R. rubrum* des PD.

Surtout, les CE50 obtenues avec les fractions F2 ont été plus basses que celles acquises avec les F1. De même, de manière général, les fractions de type PD ont été plus efficaces que celles où les PC dominaient. Les résultats des fractions évaluées sur la souche Juan ont montrée des CE50 plus basses que celles sur la souche INRA. Telles résultats suggèrent une plus sensibilité de la souche Juan. Pour les monomères de flavonoïdes, l'arbutine, l'acide vanillique ou la taxifoline n'ont montré aucune activité anthelminthique. A l'inverse la naringenine, la quercetine et la luteoline ont montré une réelle efficacité lors du test « LEIA ».

Par ailleurs, les tests d'évaluation des fractions de TCs en combinaison avec la quercetine ou la luteoline, ont montré des effets synergiques entre TCs et ces deux monomères flavonoïdes, puisque les valeurs des CE50 étaient réduites, par comparaison à celles mesurées sans flavonoïdes. L'effet synergique a été plus fort pour les TCs du type PC. Cette étude est le premier rapport suggérant un synergisme d'activité AH contre les nématodes gastro-intestinaux entre TCs et des flavonoïdes.

International Journal for Parasitology: Drugs and Drug Resistance 5 (2015) 127–134

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijppaw

Synergistic inhibition of *Haemonchus contortus* exsheathment by flavonoid monomers and condensed tannins

Chaweewan Klongsiriwet [a, *], Jessica Quijada [b], Andrew R. Williams [c], Irene Mueller-Harvey [a], Elizabeth M. Williamson [d], Hervé Hoste [b]

[a] *Chemistry and Biochemistry Laboratory, School of Agriculture, Policy and Development, University of Reading, P O Box 236, Reading RG6 6AT, UK*
[b] *UMR IHAP 1225 INRA/ENVT, Ecole Nationale Veterinaire de Toulouse, 31076, 23 Chemin des Capelles, Toulouse Cedex, France*
[c] *Faculty of Health and Medical Sciences, University of Copenhagen, 1871 Frederiksberg C, Denmark*
[d] *School of Pharmacy, P O Box 226, Whiteknights, Reading RG6 6AP, UK*

ARTICLE INFO

Article history:
Received 23 April 2015
Received in revised form
15 June 2015
Accepted 16 June 2015
Available online xxx

Keywords:
Nematodes
Procyanidins
Prodelphinidins
Proanthocyanidins
Mean degree of polymerisation
Flavan-3-ols
Quercetin
Luteolin

ABSTRACT

This study investigated the separate and combined anthelmintic (AH) effects of different phenolic compounds, including condensed tannins and flavonoids, all of which are known to occur in willow leaves, a potentially valuable dry season feed. A range of contrasting model tannins, which span the whole range of willow tannins, were isolated from tilia flowers, goat willow leaves, black currant leaves and red currant leaves. All together, the tested compounds represented the major tannin types (procyanidins and prodelphinidins) and flavonoid types (flavonols, flavones and flavanones). The larval exsheathment inhibition assay (LEIA) was used to assess their *in vitro* effects on *Haemonchus contortus* third stage larvae. Arbutin, vanillic acid, and taxifolin proved to be ineffective whereas naringenin, quercetin and luteolin were highly effective at 250 μM concentrations. Procyanidin (PC) tannins tended to be less active than prodelphinidin tannins (PD). Experiments with combinations of tannins and quercetin or luteolin revealed for the first time the existence of synergistic AH effects between tannins and flavonoid monomers. They also provided evidence that synergistic effects appear to occur at slightly lower concentrations of PC than PD. This suggests that the AH activity of condensed tannins can be significantly enhanced by the addition of quercetin or luteolin. This information may prove useful for plant breeding or selection and for designing optimal feed mixtures.

1. Introduction

Infection of small ruminants with gastrointestinal nematodes (GINs) remains a serious pathological problem across the world affecting animal health, welfare and production, as there is now a critically high level of drug resistance (Jackson et al., 2012). These GINs are able to develop resistance to new synthetic (anthelmintic, AH) drugs within a few years (Waller, 2006) and, therefore, sustainable livestock farming can no longer rely on deworming with AH drugs. New approaches for the sustainable control of GIN seek to lower parasite numbers to a manageable level or to modify their biological development and life cycle, rather than to eliminate them completely. Medicinal plants have been used since antiquity to treat worm infections (Hrckova and Velebny, 2013) and antiparasitic properties of plants against GIN have been linked to the presence of proteinases (Stepek et al., 2004) and other secondary plant metabolites. Plant phenolics, flavonoids and condensed tannins have received considerable attention over the last few years (Barrau et al., 2005; Hoste et al., 2006, 2012) as plants that produce these compounds grow in all regions of the world. Condensed tannins (CT; *syn.* proanthocyanidins) can have direct and indirect AH effects (Min et al., 2003; Hoste et al., 2012), as well as having the potential to enhance the host's innate immune response (Tibe et al., 2012). The hypothesis for direct effects of polyphenolic compounds has been substantiated by several *in vitro* assays against different life-cycle stages (Bahuaud et al., 2006; Novobilský et al., 2011).

Condensed tannins are oligomers or polymers of flavan-3-ols and are classified into different subgroups. The two major CT types are procyanidins (PC), which have two OH-groups, and prodelphinidins (PD), which have three OH-groups in the B-ring (Fig. 1). The stereochemistry at the heterocyclic C-ring gives rise to

---

* Corresponding author.
E-mail addresses: c.klongsiriwet@student.reading.ac.uk, kchaweew@wu.ac.th (C. Klongsiriwet).

http://dx.doi.org/10.1016/j.ijpddr.2015.06.001

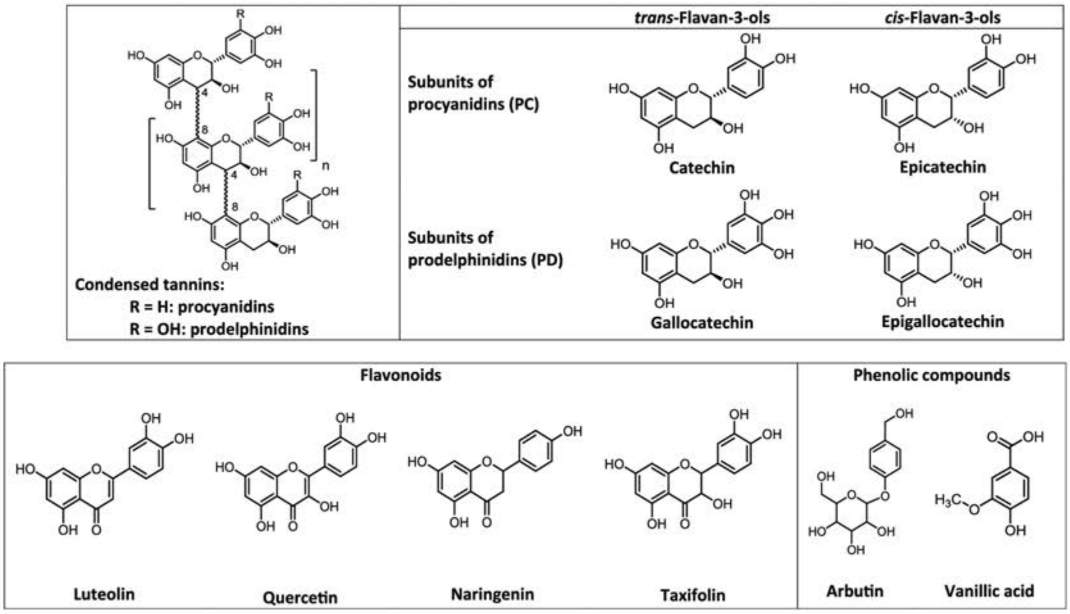


Fig. 1. Structures of condensed tannins, flavonoids (flavan-3-ols, luteolin, quercetin, naringenin and taxifolin) and other polyphenols (arbutin and vanillic acid).

either 2–3 *cis*- or 2–3 *trans*-flavan-3-ols and the average polymer length of CT mixtures is described in terms of mean degree of polymerisation (mDP). It is important to note that most condensed tannin-containing forages tend to contain complex mixtures of PC and PD homo- and hetero-polymers that are difficult to separate. However, a few plants specialise in the synthesis of either PCs or PDs and can thus serve as valuable sources for the different tannin types and as research tools for probing structure–activity relationships.

Since tannins and other flavonoids co-occur in plants, it is pertinent to investigate their combined potencies. To this end, we selected polyphenols, which are known to occur in willow (*Salix* spp, Salicaceae), as this species represents a potentially valuable nutraceutical forage (Moore et al., 2003; Diaz Lira et al., 2008; Mupeyo et al., 2011). Moreover, breeding programmes are currently focussing on increasing the *Salix* wood production as a renewable source of energy (Karp et al., 2011) and, therefore, it is timely to explore, which phenolic compositions can contribute most to the AH properties of the leaf by-products. The phenolic composition of willow leaves has been studied already by several groups (Jarrett and Williams, 1967; Pohjamo et al., 2003; Enayat and Banerjee, 2009) and we recently reported that willow tannins cover a wide range of PC/PD ratios (from 15/85 to 98/2) and *cis/trans* flavan-3-ol ratios (from 2/98 to 81/19) (Falchero et al., 2011). We now describe the isolation of distinct PC and PD types from specialist CT plant sources in order to cover the full spectrum of *Salix* tannins and tested their AH effects in the presence of commercially available phenolic monomers that are known to be present in *Salix* leaves. This allowed us to test two hypotheses; the first stated that CT and other naturally occurring polyphenolic monomers exert synergistic inhibitory effects on the *in vitro* L3 exsheathment of *Haemonchus contortus* and the second that these AH interactions depend on CT types.

2. Materials and methods

2.1. Chemicals and plant materials

Arbutin, luteolin and taxifolin were purchased from Apin Chemicals Ltd (Abingdon, U.K.); vanillic acid and naringenin from Alfa Aesar (Blackpool, U.K.); gallocatechin and quercetin from Sigma–Aldrich (Gillingham, U.K.); phosphate buffered saline (PBS) from bioMerieux (Lyon, France), Milton solution, sodium hypochlorite 2% w/v and sodium chloride 16.5% w/v from Milton (Inibsa laboratorios, Barcelona, Spain). Goat willow (*Salix caprea*), black currant (*Ribes nigrum*) and red currant (*Ribes rubrum*) leaves were collected in July and August 2012. Tilia (Linden) flowers (*Tilia* × *europaea*) were purchased from Flos (Mokrsko, Poland). These plant samples were chosen as they allowed the testing of different tannin types, all of which are known to occur in willow.

2.2. Preparation of plant extracts and tannin fractions

Freeze-dried, powdered plant material (25 g of each species) was extracted with an acetone:water mixture (7:3, v/v; 300 ml) (Williams et al., 2014a) and filtered. The filtrate was extracted with dichloromethane (250 ml) and the organic phase discarded. The aqueous phase was rotary evaporated under vacuum at 40 °C to remove residual organic solvents and then lyophilised. The freeze-dried extract was re-dissolved in distilled water, filtered under vacuum to remove insoluble particles and applied to a Sephadex LH-20 column. Fraction 1 was eluted with acetone/water (3:7, v/v) and fraction 2 with acetone/water (1:1, v/v). Acetone was removed in a rotary evaporator and the aqueous residue freeze-dried. Tannins were quantified and characterized by thiolysis with benzylmercaptan (Gea et al., 2011). Tannin composition was determined in terms of the percentage of PCs and PDs, *cis*- and *trans*-flavan-3-ol subunits and mean degree of polymerisation (mDP), which were calculated according to Gea et al. (2011).

2.3. Bioassays

2.3.1. Haemonchus contortus strains

Third-stage larvae (L3) were obtained from sheep or goats infected with two monospecific strains of *H. contortus* that were susceptible to AH drugs (Alonso-Díaz et al., 2008). The Juan strain was obtained from donor sheep, which were kept indoors and infected monospecifically with *H. contortus*, and the larvae had been maintained in the laboratory for four months. This strain was used initially to investigate the effects of four F1- and four F2-tannin fractions and of several phenolic compounds. The INRA strain was obtained from monospecifically infected goats and the larvae had been maintained in the laboratory for 1 month before use. This strain was used to repeat experiments with the F2 tannin fractions, two of the flavonoids (quercetin and luteolin) and also with the tannin-flavonoid mixtures. The facilities hosting the animals and the trial was performed according to French ethical and welfare rules (agreement number C 31 555 27 of 19 August 2010).

2.3.2. Larval exsheathment inhibition assay (LEIA)

The LEIA was performed as described previously (Bahuaud et al., 2006). Briefly, third-stage larvae were incubated with the tannin or flavonoid test solutions at 23 °C for 3 h, washed with PBS and centrifuged 3 times. The pelleted larvae were suspended in 200 μl water (ca 360–420 nematode L3 larvae) and 40 μl were removed to count the proportion of exsheathed larvae at 0 min. The remaining larvae (160 μl) were then subjected to the artificial exsheathment process by adding 40 μl of a Milton solution (2% w/v sodium hypochlorite and 16.5% sodium chloride) diluted in PBS, where the Milton:PBS ratio varied from 1 in 250 to 1 in 330 (this ratio was tested at the start of each experiment per batch in order to ensure 100% exsheathment within 60 min in the negative control). The numbers of ensheathed and exsheathed larvae were counted under a microscope at 0, 20, 40 and 60 min after contact with the Milton solution to investigate the proportion of exsheathed larvae over time. The tannin fractions were prepared as final serial concentrations of 600, 300, 150, 75, 37.5 μg fractions/ml and all other phenolic compounds at 1000, 500, 250, 100, 60 μM in PBS (0.1 M phosphate, 0.05 M NaCl, pH 7.2). The experiments with tannin-flavonoid mixtures were conducted using mixed solutions containing the same final concentrations of F2 fractions (600, 300, 150, 75, 37.5 μg fractions/ml) and combined with a fixed flavonoid concentration (either 60 or 30 μM quercetin or luteolin) in PBS. Four replicates were performed for each test solution and the negative control, L3 in PBS, was run in parallel.

2.4. Statistical analysis

The 50% effective concentration (EC_{50}-value) was calculated using PoloPlus© (version 1.0; LeOra Software Company, Petaluma, California, USA). These EC_{50}-values are reported on the basis of tannin content in the F1 and F2 fractions (see Table 1). To assess possible synergistic effects of the tannin/flavonoid combinations, we used the observed inhibitory effects of the individual treatments to calculate the additive inhibitory effect that would be predicted to be achieved by the mixture under the assumption of independent action, using the definition of Bliss additivity (Bliss, 1939). The observed effect of the mixture was then compared to this predicted additive effect, with deviations from the predicted values indicating either synergy (greater effect) or antagonism (lower effect) (Williams et al., 2012).

3. Results

3.1. Condensed tannin analysis

The tannin fractions from the four plant species differed significantly in composition as shown in Table 1. The CT contents ranged from 43.4 to 99.8 g CT/100 g fraction; the mean degree of polymerisation (mDP) from 2.3 to 19.0; the tannin PC/PD ratios from 5.1/94.9 to 100/0 and the *cis/trans* flavan-3-ol ratios from 6.0/94.0 to 95.4/4.6. The fractions that eluted early from the Sephadex LH-20 column (F1 fractions) had lower CT contents (43.4–70.4 g CT/100 g fraction) and mDP-values (2.3–5.4) than the later eluting F2 fractions (CT-contents were 88.9–99.8 g CT/100 g fraction and mDP values were 6.4–19.0). Goat willow leaf and tilia flower tannins were mostly PCs, but black and red currant leaf tannins were mostly PDs. Stereochemistry added an additional distinguishing feature: red currant and tilia tannins contained predominantly *cis*-flavan-3-ols, but black currant and goat willow tannins had mostly *trans*-flavan-3-ols.

3.2. In vitro larval exsheathment inhibition by condensed tannins and other phenolic compounds

All tannin samples were first screened with four-month old larvae (Juan *H. contortus* strain from sheep) in the *in vitro* larval exsheathment inhibition assay. This experiment was then repeated with one-month old larvae (INRA *H. contortus* strain maintained on goats) and focussed only on the more potent F2 fractions. Results with the Juan strain gave 1.7–4.6-fold lower EC_{50}-values than with the INRA strain (Table 2). Further experiments will be necessary to elucidate the underlying causes of this difference, such as larval age or origin, as older larvae from sheep were more sensitive to CTs ($P < 0.05$) than younger larvae from goats.

These initial studies with the Juan strain showed that the EC_{50}-values were generally lower with the F2 tannins than the corresponding F1 fractions from each plant source (Table 2). The Table and Fig. 2 also show that the PDs from black and red (in the web version) currant leaves tended to be more active than the PCs from tilia flowers and goat willow leaves.

The phenolic monomers differed greatly in their ability to inhibit larval exsheathment: arbutin, vanillic acid and taxifolin had no activity, whereas gallocatechin showed some activity at 1000 μM. However, naringenin, quercetin and luteolin were highly effective at 250 μM. Quercetin and luteolin were chosen (at 30 and 60 μM) for further investigation in combination with the different CTs.

3.3. In vitro larval exsheathment inhibition by condensed tannin-quercetin mixtures

Preliminary experiments using mixtures of serial CT concentrations with 200 or 100 μM quercetin completely inhibited exsheathment. Therefore, much lower quercetin concentrations (60 and 30 μM) were tested in the mixture experiments. The EC_{50}-values of the mixture experiments with CTs and quercetin (30 and 60 μM) are shown in Table 2. The mixtures of PC fractions with the high quercetin concentration (60 μM) gave 3.8–5.7 fold lower EC_{50}-values than these tannin fractions on their own; at the lower quercetin concentration (30 μM) the mixture gave a 2.3 fold lower EC_{50}-value than the tilia tannin fraction. However, the mixtures of PD fractions with either quercetin concentration gave only 1.6–2.0 fold lower EC_{50}-values than tannin fractions on their own.

The inhibition profiles generated by the mixtures revealed a dose–response relationship between *H. contortus* exsheathment and CT or quercetin concentrations as illustrated for the red currant

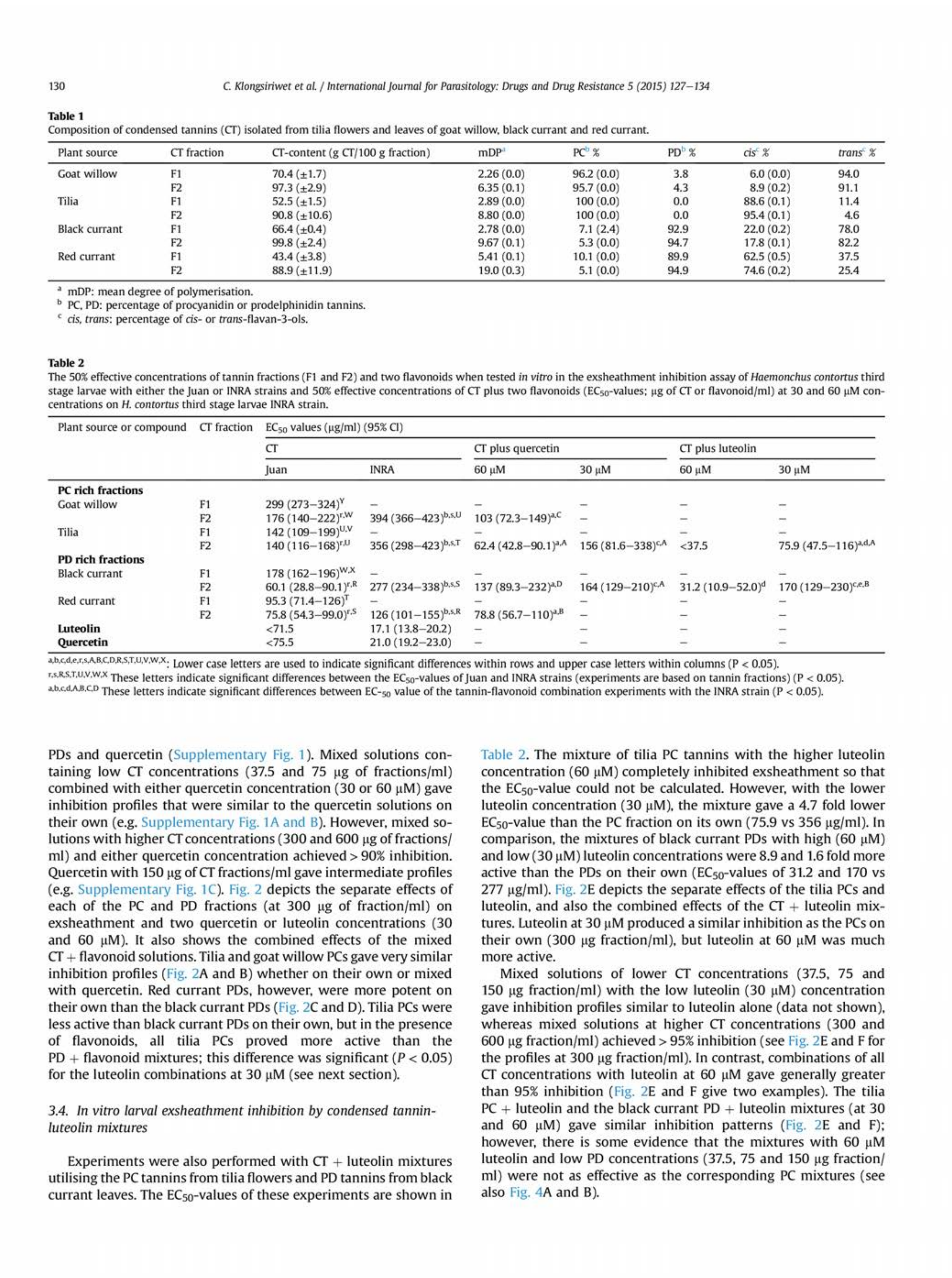


Table 1
Composition of condensed tannins (CT) isolated from tilia flowers and leaves of goat willow, black currant and red currant.

Plant source	CT fraction	CT-content (g CT/100 g fraction)	mDP[a]	PC[b] %	PD[b] %	*cis*[c] %	*trans*[c] %
Goat willow	F1	70.4 (±1.7)	2.26 (0.0)	96.2 (0.0)	3.8	6.0 (0.0)	94.0
	F2	97.3 (±2.9)	6.35 (0.1)	95.7 (0.0)	4.3	8.9 (0.2)	91.1
Tilia	F1	52.5 (±1.5)	2.89 (0.0)	100 (0.0)	0.0	88.6 (0.1)	11.4
	F2	90.8 (±10.6)	8.80 (0.0)	100 (0.0)	0.0	95.4 (0.1)	4.6
Black currant	F1	66.4 (±0.4)	2.78 (0.0)	7.1 (2.4)	92.9	22.0 (0.2)	78.0
	F2	99.8 (±2.4)	9.67 (0.1)	5.3 (0.0)	94.7	17.8 (0.1)	82.2
Red currant	F1	43.4 (±3.8)	5.41 (0.1)	10.1 (0.0)	89.9	62.5 (0.5)	37.5
	F2	88.9 (±11.9)	19.0 (0.3)	5.1 (0.0)	94.9	74.6 (0.2)	25.4

[a] mDP: mean degree of polymerisation.
[b] PC, PD: percentage of procyanidin or prodelphinidin tannins.
[c] *cis*, *trans*: percentage of *cis*- or *trans*-flavan-3-ols.

Table 2
The 50% effective concentrations of tannin fractions (F1 and F2) and two flavonoids when tested *in vitro* in the exsheathment inhibition assay of *Haemonchus contortus* third stage larvae with either the Juan or INRA strains and 50% effective concentrations of CT plus two flavonoids (EC_{50}-values; µg of CT or flavonoid/ml) at 30 and 60 µM concentrations on *H. contortus* third stage larvae INRA strain.

Plant source or compound	CT fraction	EC_{50} values (µg/ml) (95% CI)					
		CT		CT plus quercetin		CT plus luteolin	
		Juan	INRA	60 µM	30 µM	60 µM	30 µM
PC rich fractions							
Goat willow	F1	299 (273–324)^{Y}	–	–	–	–	–
	F2	176 (140–222)^{r,W}	394 (366–423)^{b,s,U}	103 (72.3–149)^{a,C}	–	–	–
Tilia	F1	142 (109–199)^{U,V}	–	–	–	–	–
	F2	140 (116–168)^{r,U}	356 (298–423)^{b,s,T}	62.4 (42.8–90.1)^{a,A}	156 (81.6–338)^{c,A}	<37.5	75.9 (47.5–116)^{a,d,A}
PD rich fractions							
Black currant	F1	178 (162–196)^{W,X}	–	–	–	–	–
	F2	60.1 (28.8–90.1)^{r,R}	277 (234–338)^{b,s,S}	137 (89.3–232)^{a,D}	164 (129–210)^{c,A}	31.2 (10.9–52.0)^{d}	170 (129–230)^{c,e,B}
Red currant	F1	95.3 (71.4–126)^{T}	–	–	–	–	–
	F2	75.8 (54.3–99.0)^{r,S}	126 (101–155)^{b,s,R}	78.8 (56.7–110)^{a,B}	–	–	–
Luteolin		<71.5	17.1 (13.8–20.2)	–	–	–	–
Quercetin		<75.5	21.0 (19.2–23.0)	–	–	–	–

[a,b,c,d,e,r,s,A,B,C,D,R,S,T,U,V,W,X]: Lower case letters are used to indicate significant differences within rows and upper case letters within columns ($P < 0.05$).
[r,s,R,S,T,U,V,W,X] These letters indicate significant differences between the EC_{50}-values of Juan and INRA strains (experiments are based on tannin fractions) ($P < 0.05$).
[a,b,c,d,A,B,C,D] These letters indicate significant differences between EC-$_{50}$ value of the tannin-flavonoid combination experiments with the INRA strain ($P < 0.05$).

PDs and quercetin (Supplementary Fig. 1). Mixed solutions containing low CT concentrations (37.5 and 75 µg of fractions/ml) combined with either quercetin concentration (30 or 60 µM) gave inhibition profiles that were similar to the quercetin solutions on their own (e.g. Supplementary Fig. 1A and B). However, mixed solutions with higher CT concentrations (300 and 600 µg of fractions/ml) and either quercetin concentration achieved > 90% inhibition. Quercetin with 150 µg of CT fractions/ml gave intermediate profiles (e.g. Supplementary Fig. 1C). Fig. 2 depicts the separate effects of each of the PC and PD fractions (at 300 µg of fraction/ml) on exsheathment and two quercetin or luteolin concentrations (30 and 60 µM). It also shows the combined effects of the mixed CT + flavonoid solutions. Tilia and goat willow PCs gave very similar inhibition profiles (Fig. 2A and B) whether on their own or mixed with quercetin. Red currant PDs, however, were more potent on their own than the black currant PDs (Fig. 2C and D). Tilia PCs were less active than black currant PDs on their own, but in the presence of flavonoids, all tilia PCs proved more active than the PD + flavonoid mixtures; this difference was significant ($P < 0.05$) for the luteolin combinations at 30 µM (see next section).

3.4. *In vitro larval exsheathment inhibition by condensed tannin-luteolin mixtures*

Experiments were also performed with CT + luteolin mixtures utilising the PC tannins from tilia flowers and PD tannins from black currant leaves. The EC_{50}-values of these experiments are shown in Table 2. The mixture of tilia PC tannins with the higher luteolin concentration (60 µM) completely inhibited exsheathment so that the EC_{50}-value could not be calculated. However, with the lower luteolin concentration (30 µM), the mixture gave a 4.7 fold lower EC_{50}-value than the PC fraction on its own (75.9 vs 356 µg/ml). In comparison, the mixtures of black currant PDs with high (60 µM) and low (30 µM) luteolin concentrations were 8.9 and 1.6 fold more active than the PDs on their own (EC_{50}-values of 31.2 and 170 vs 277 µg/ml). Fig. 2E depicts the separate effects of the tilia PCs and luteolin, and also the combined effects of the CT + luteolin mixtures. Luteolin at 30 µM produced a similar inhibition as the PCs on their own (300 µg fraction/ml), but luteolin at 60 µM was much more active.

Mixed solutions of lower CT concentrations (37.5, 75 and 150 µg fraction/ml) with the low luteolin (30 µM) concentration gave inhibition profiles similar to luteolin alone (data not shown), whereas mixed solutions at higher CT concentrations (300 and 600 µg fraction/ml) achieved > 95% inhibition (see Fig. 2E and F for the profiles at 300 µg fraction/ml). In contrast, combinations of all CT concentrations with luteolin at 60 µM gave generally greater than 95% inhibition (Fig. 2E and F give two examples). The tilia PC + luteolin and the black currant PD + luteolin mixtures (at 30 and 60 µM) gave similar inhibition patterns (Fig. 2E and F); however, there is some evidence that the mixtures with 60 µM luteolin and low PD concentrations (37.5, 75 and 150 µg fraction/ml) were not as effective as the corresponding PC mixtures (see also Fig. 4A and B).

3.5. Synergistic effects between condensed tannins and quercetin

The CT-quercetin mixtures revealed synergistic effects with CTs. Fig. 3 shows that the observed inhibition was much greater than the predicted additive inhibition calculated from the separate CT and quercetin data points (i.e. predicted additive effect): synergy was demonstrated between quercetin and tilia PCs (Fig. 3A) at 150 and 300 μg fraction/ml after 60 min, and with goat willow PCs (Fig. 3C) at 300 μg fraction/ml. Synergy was also observed with black currant PDs at 300 μg fraction/ml (Fig. 3B) and red currant PDs at 150 and 300 μg fraction/ml (Fig. 3D). In addition, synergy could be detected at 40 min but at a slightly lower level (not shown).

3.6. Synergistic effects between condensed tannins and luteolin

Two of the slightly weaker AH tannins, tilia PCs and black currant PDs, were also evaluated for synergy at two luteolin concentrations. Fig. 4 shows the effects after 60 min. In the presence of 60 μM luteolin, tilia PCs exhibited clear synergy at all concentrations, but black currant PDs showed synergy only at 300 μg fraction/ml. At the lower luteolin concentration (30 μM), tilia PCs acted synergistically at 300 μg fraction/ml and black currant PDs at 150 and 300 μg fraction/ml.

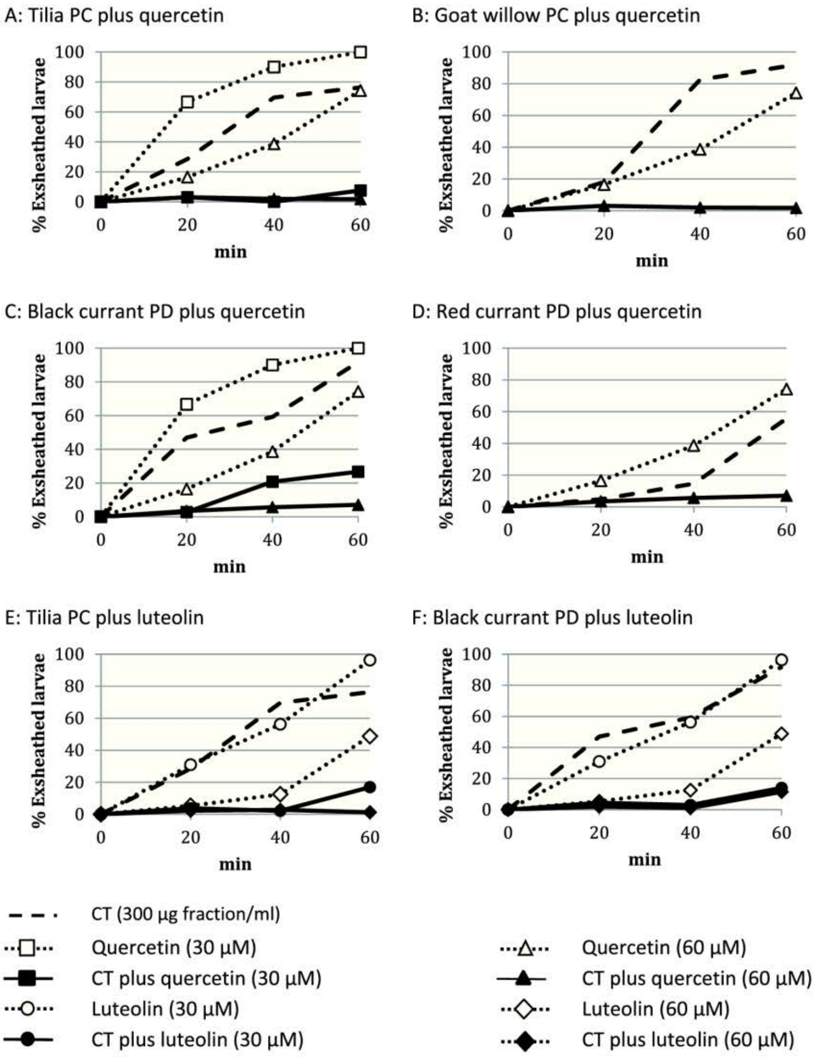


Fig. 2. Larval exsheathment of *Haemonchus contortus* in the presence of different condensed tannin types (at 300 μg fraction/ml), which were isolated from tilia flowers, goat willow leaves, black currant leaves and red currant leaves, and quercetin or luteolin (30 and 60 μM) and their combinations (CT = condensed tannins; PC = procyanidin tannins; PD = prodelphinidin tannins).

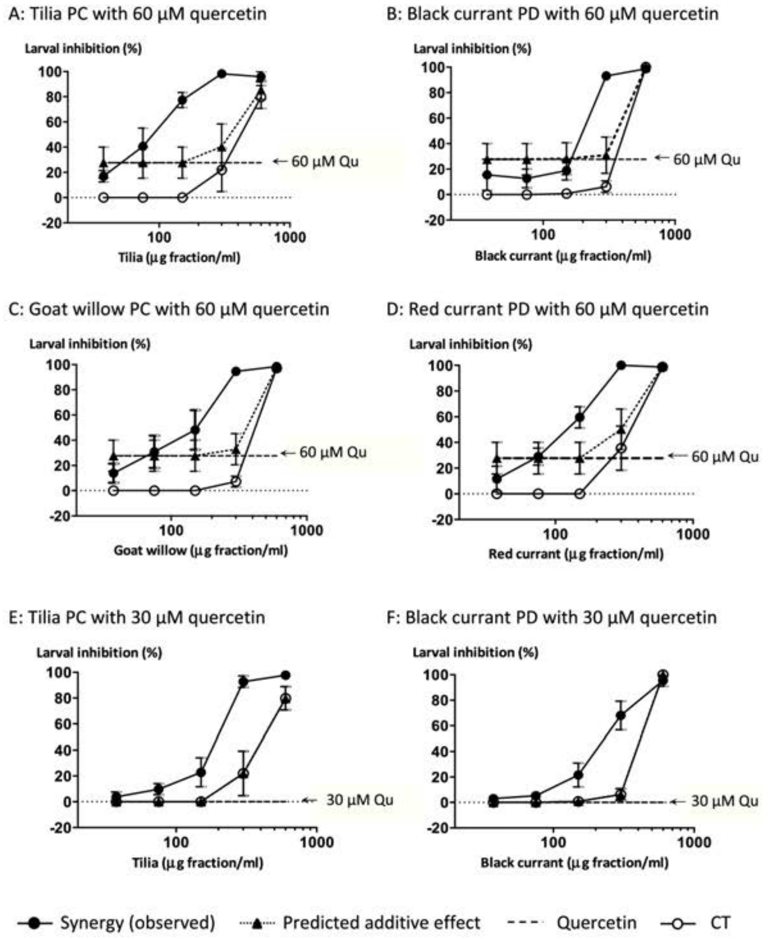


Fig. 3. Synergistic effects between condensed tannins (CT) and quercetin (60 and 30 μM) on the inhibition of *Haemonchus contortus* larval exsheathment after 60 min.

4. Discussion

We chose the LEIA as it has advantages over other bioassays in that it is sensitive, reproducible and relevant to processes that occur *in vivo* (Alonso-Diaz et al., 2011; Azando et al., 2011). This assay was, therefore, chosen for evaluating the different CTs and flavonoids. The main aim was to investigate their combined effects as tannin-containing plants usually contain mixtures of CTs and other flavonoids.

PCs and PDs are the most common condensed tannins in forages and browse plants (Marais et al., 2000; Tibe et al., 2011) and tend to occur as complex combinations of homo- and hetero-polymers (Stringano et al., 2012) that are difficult to separate. However, well-defined tannin types are needed for unravelling the relationships between tannin structures and AH activities. A more straightforward approach, therefore, is to exploit plants that specialise in producing relatively pure but different tannin types (Williams et al., 2014a). The ultimate aim of this strategy is to provide plant breeders with targets for enhancing the nutraceutical properties of new animal feeds or varieties. Thus we isolated tannins as relatively pure homopolymers of 95.7–100% PCs and 89.9–94.7% PDs (Table 1) and also obtained two procyanidin subsets and two prodelphinidin subsets with low and high *cis/trans* flavan-3-ol ratios. Quercetin and luteolin are widely distributed in plants (Yang et al., 2008; López-Lázaro, 2009) and represent two important flavonoid subgroups, i.e. flavonols and flavones, respectively (Fig. 1), and were thus chosen for the combination experiments with CTs. Initial studies tested the AH effects of the four tannin types at several concentrations. Whilst the results confirmed that PDs tended to be better anthelmintics than PCs (Min and Hart, 2003; Brunet and Hoste, 2006; Novobilský et al., 2011), little is known about whether stereochemistry of the flavan-3-ol subunits has any effect. Our data provided an indication that the *cis/trans* stereochemistry of the flavan-3-ol subunits did not impact

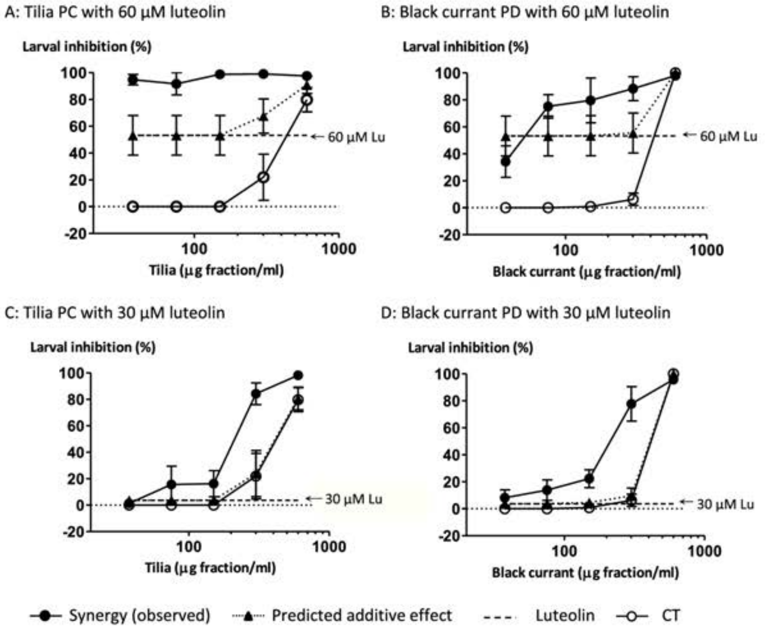


Fig. 4. Synergistic effects between condensed tannins (CT) and luteolin (60 and 30 μM) on the inhibition of *Haemonchus contortus* larval exsheathment after 60 min.

on AH activities (Table 1) in agreement with a previous study (Williams et al., 2014a), although further work will be needed as the average polymer sizes also varied for the PD subset (mDP-values were 10 and 19).

The data showed that tannin concentrations, which ranged from 43.4 to 99.8 g CT/100 g fraction, were not correlated with EC_{50} values. This points to the possibility that 'impurities' in these fractions may have moderated tannin activities. Typical impurities in aqueous acetone extracts and partially purified tannin fractions tend to consist of low molecular weight phenolics and flavonoids (Tibe et al., 2011).

Given that we observed increased inhibition of exsheathment with a mixture of tannins and quercetin or luteolin, we next quantified whether this effect was additive or synergistic in nature. Synergy refers to phenomena where two or more agents together produce an effect greater than would be predicted from their individual contributions. There are various mathematical models available to assess synergy, all of which have advantages and disadvantages (Williamson, 2001). Using the Bliss model (Williams et al., 2012), we observed synergistic effects between all CTs and quercetin or luteolin in terms of AH activity during the exsheathment of 3rd stage *H. contortus* larvae (Figs. 3 and 4). These CTs were tested at serial concentrations of 37.5, 75, 150, 300, 600 μg fraction/ml, which are physiologically relevant as extractable tannins range from 350 to 900 μg/ml in the sheep gut (Terrill et al., 1994; Barrau et al., 2005). However, no such information was found for quercetin or luteolin. It would seem that the AH effects of tilia PCs were slightly more enhanced, especially at lower concentrations, by quercetin or luteolin than the AH effects of PDs. A likely explanation could be that PCs offer more scope for enhancement as they tend to have weaker AH activities than PDs (Brunet and Hoste, 2006; Novobliský et al., 2011).

The mechanism(s) of the observed synergistic actions are not yet known. Tannins have been shown to deform nematode surfaces (Hoste et al., 2006; Williams et al., 2014a, b). Interestingly, exposure to extracts from tannin-containing plants (which would also have contained flavonoids) caused degeneration of muscle cells, accumulation of electron-dense vesicles and marked intracellular disorganisation (Brunet et al., 2011). These authors speculated whether the lesions observed in the cuticle of ensheathed L3 larvae could have been due to accumulation of metabolic products, which in turn could have produced cellular toxicity by blocking the metabolic exchange with the environment. Quercetin is well known as an inhibitor of P-glycoproteins (P-gp), which play an important role in the transport of xenobiotics (Lespine et al., 2012). It is not known whether this activity also contributes to enhancing the anthelmintic effects of tannins.

To summarise, this study provided the first evidence of synergistic effects between condensed tannins and two common flavonoids, quercetin and luteolin, in terms of inhibiting the *in vitro* exsheathment of *H. concortus* L3 larvae. Some of the synergistic effects occurred at lower PC than PD concentrations, perhaps implying that the generally weaker AH activity of PCs can benefit more from the addition of quercetin. These findings suggest that opportunities should be investigated for increasing anthelmintic activity by mixing plant materials that contain condensed tannins and quercetin or luteolin flavonoids, or to select plants with enhanced tannin and quercetin or luteolin contents.

Conflicts of interest

The authors declared that there is no conflict of interest.

Acknowledgements

C. Klongsiriwet is grateful to the Royal Thai Government for a PhD scholarship. Hildred PYO-Farm (Goring-on-Thames, Reading, U.K.) kindly provided leaves from black and red currant bushes and

Mr and Mrs D. and A. Prudence (Goring-on-Thames, Oxfordshire, U.K.) provided the goat willow (*Salix caprea*) leaves. J. Quijada is grateful for financial support from the European Commission (Marie Curie Initial Training Network, PITN-GA-2011-289377, “LegumePlus”).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijpddr.2015.06.001.

References

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Aguilar-Caballero, A.J., Hoste, H., 2008. *In vitro* larval migration and kinetics of exsheathment of *Haemonchus contortus* larvae exposed to four tropical tanniniferous plant extracts. Vet. Parasitol. 153, 313–319.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2011. Comparing the sensitivity of two *in vitro* assays to evaluate the anthelmintic activity of tropical tannin rich plant extracts against *Haemonchus contortus*. Vet. Parasitol. 181, 360–364.

Azando, E.V., Hounzangbe-Adote, M.S., Olounlade, P.A., Brunet, S., Fabre, N., Valentin, A., Hoste, H., 2011. Involvement of tannins and flavonoids in the *in vitro* effects of *Newbouldia laevis* and *Zanthoxylum zanthoxyloides* extracts on the exsheathment of third-stage infective larvae of gastrointestinal nematodes. Vet. Parasitol. 180, 292–297.

Bahuaud, D., Martinez-Ortiz de Montellano, C., Chauveau, S., Prevot, F., Torres-Acosta, F., Fouraste, I., Hoste, H., 2006. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. Parasitology 132, 545–554.

Barrau, E., Fabre, N., Fouraste, I., Hoste, H., 2005. Effect of bioactive compounds from sainfoin (*Onobrychis viciifolia*) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. Parasitology 131, 531–538.

Bliss, C.I., 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26, 585–615.

Brunet, S., Hoste, H., 2006. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. J. Agric. Food Chem. 54, 7481–7487.

Brunet, S., Fourquaux, I., Hoste, H., 2011. Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (*Onobrychis viciifolia*) extract. Parasitol. Int. 60, 419–424.

Diaz Lira, C.M., Barry, T.N., Pomroy, W.E., McWilliam, E.L., Lopez-Villalobos, N., 2008. Willow (*Salix* spp.) fodder blocks for growth and sustainable management of internal parasites in grazing lambs. Anim. Feed Sci. Tech. 141, 61–81.

Enayat, S., Banerjee, S., 2009. Comparative antioxidant activity of extracts from leaves, bark and catkins of *Salix aegyptiaca* sp. Food Chem. 116, 23–28.

Falchero, L., Brown, R.H., Karp, A., Hanley, S., Shield, I., Mueller-Harvey, I., 2011. The structural diversity of condensed tannins in willows (*Salix* spp.): a first screening to improve the nutritional quality of ruminant products. Adv. Anim. Biosci. 2, 410.

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey, I., 2011. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. J. Agric. Food Chem. 59, 495–503.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M., Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol. 22, 253–261.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F., Sandoval-Castro, C.A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. Vet. Parasitol. 186, 18–27.

Hrckova, G., Velebny, S., 2013. Parasite helminths of humans and animals: health impact and control. In: Hrckova, G., Velebny, S. (Eds.), Pharmacological Potential of Selected Natural Compounds in the Control of Parasitic Diseases. Springer-Verlag, Wien, Austria, pp. 29–99.

Jackson, F., Varady, M., Bartley, D.J., 2012. Managing anthelmintic resistance in goats—Can we learn lessons from sheep? Small Rumin. Res. 103, 3–9.

Jarrett, J.M., Williams, A.H., 1967. The flavonoid glycosides of *Salix purpurea*. Phytochemistry 6, 1585–1586.

Karp, A., Hanley, S.J., Trybush, S.O., Macalpine, W., Pei, M., Shield, I., 2011. Genetic improvement of willow for bioenergy and biofuels. J. Integr. Plant Biol. 53, 151–165.

Lespine, A., Ménez, C., Bourguinat, C., Prichard, R.K., 2012. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: prospects for reversing transport-dependent anthelmintic resistance. Int. J. Parasitol. Drugs Drug Resist 2, 58–75.

López-Lázaro, M., 2009. Distribution and biological activities of the flavonoid luteolin. Mini-Rev. Med. Chem. 9, 31–59.

Marais, J.P.J., Mueller-Harvey, I., Brandt, E.V., Ferreira, D., 2000. Polyphenols, condensed tannins and other natural products in *Onobrychis viciifolia* (sainfoin). J. Agric. Food Chem. 48, 3440–3447.

Min, B.R., Barry, T.N., Attwood, G.T., McNabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. Anim. Feed Sci. Tech. 106, 3–19.

Min, B.R., Hart, S.P., 2003. Tannins for suppression of internal parasites. J. Anim. Sci. 81 (E. Suppl. 2), E102–E109.

Moore, K.M., Barry, T.N., Cameron, P.N., Lopez-Villalobos, N., Cameron, D.J., 2003. Willow (*Salix* sp.) as a supplement for grazing cattle under drought conditions. Anim. Feed Sci. Tech. 104, 1–11.

Mupeyo, B., Barry, T.N., Pomroy, W.E., Ramírez-Restrepo, C.A., López-Villalobos, N., Pernthaner, A., 2011. Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. Anim. Feed Sci. Tech. 164, 8–20.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. Vet. Parasitol. 182, 213–220.

Pohjamo, S.P., Hemming, J.E., Willför, S.M., Reunanen, M.H.T., Holmbom, B.R., 2003. Phenolic extractives in *Salix caprea* wood and knots. Phytochemistry 63, 165–169.

Stepek, G., Behnke, J.M., Buttle, D.J., Duce, I.R., 2004. Natural plant cysteine proteinases as anthelmintics. Trends Parasitol. 7, 322–327.

Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Brown, R.H., Mueller-Harvey, I., 2012. Proanthocyanidin diversity in the EU ‘HealthyHay’ sainfoin (*Onobrychis viciifolia*) germplasm collection. Phytochemistry 77, 197–208.

Terrill, T.H., Waghorn, G.C., Woolley, D.J., McNabb, W.C., Barry, T.N., 1994. Assay and digestion of 14C-labelled condensed tannins in the gastrointestinal tract of sheep. Br. J. Nutr. 72, 467–477.

Tibe, O., Meagher, L.P., Fraser, K., Harding, D.R.K., 2011. Condensed tannins and flavonoids from the forage legume sulla (*Hedysarum coronarium*). J. Agric. Food Chem. 59, 9402–9409.

Tibe, O., Pernthaner, A., Sutherland, I., Lesperance, L., Harding, D., 2012. Condensed tannins from Botswanan forage plants are effective priming agents of $\gamma\delta$ T cells in ruminants. Vet. Immunol. Immunopathol. 146, 237–244.

Waller, P.J., 2006. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. Vet. Parasitol. 139, 1–14.

Williams, A.R., Douglas, A.D., Miura, K., Illingworth, J.J., Choudhary, P., Murungi, L.M., Furze, J.M., Diouf, A., Miotto, O., Crosnier, C., Wright, G.J., Kwiatkowski, D.P., Fairhurst, R.M., Long, C.A., Draper, S.J., 2012. Enhancing blockade of *Plasmodium falciparum* erythrocyte invasion: assessing combinations of antibodies against PfRH5 and other merozoite antigens. PLoS Pathog. 8 (11), e1002991. http://dx.doi.org/10.1371/journal.ppat.1002991.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014a. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. PLoS ONE 9 (5), e97053. http://dx.doi.org/10.1371/journal.pone.0097053.

Williams, A.R., Ropiak, H.M., Fryganas, C., Desrues, O., Mueller-Harvey, I., Thamsborg, S.M., 2014b. Assessment of the anthelmintic activity of medicinal plant extracts and purified condensed tannins against free-living and parasitic stages of *Oesophagostomum dentatum*. Parasit. Vectors 7, 518–527.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. Phytomedicine 8, 401–409.

Yang, R.-Y., Lin, S., Kuo, G., 2008. Content and distribution of flavonoids among 91 edible plant species. Asia Pac. J. Clin. Nutr. 17 (S1), 275–279.

CHAPITRE 3

CHAPITRE 3 : Etude *in vivo* des relations structure/activité des tanins condensés sur les nématodes chez les petits ruminants

ARTICLE 3 : Comparaison des effets anthelminthiques *in vivo* de deux ressources contenant des tanins condensés différant par leur qualité

QUIJADA J., GAUDIN, E., EL-KORSO, R., RAMSAY, A. MUELLER-HARVEY, I., et HOSTE, H.

Manuscrit en préparation

RESUME – ARTICLE 3

Introduction

L'utilisation des plantes ou de ressources contenant des tanins condensés (TC), est une méthode alternative dans la maîtrise des infestations par des nématodes gastro-intestinaux (NGI) chez les ruminants. Néanmoins, des variabilités d'activité anthelminthique (AH) ont été rapportées *in vitro*. Ces résultats suggèrent qu'à la fois la quantité et la qualité (structure) des tannins condensés pourraient jouer un rôle dans l'activité AH. Toutefois, les études pour vérifier si ces facteurs influent sur les effets antheminthiques in vivo restent rares.

Dans ce but, deux ressources [le sainfoin (*Onobrychis viciifolia*) et les pellicules de noisettes (*Corylus avellana*)] contenant toutes deux des TCs avec des caractéristiques structurelles divergentes en terme de ratio prodelphinidïnes/procyanidïnes (PD/PC) mais aussi de présence de tannins avec des substituts galloyl ont été donnés à des agneaux infestés expérimentalement par *Haemonchus contortus* et *Trichostrongylus colubriformis.* Les mesures réalisées ont visé à évaluer les effets sur la biologie des populations de vers et sur la résilience de l'hôte.

Matériel et méthodes

Trois lot de 9 agneaux (n= 27) ont été infestés expérimentalement avec *H. contortus* et *T. colubriformis* à J0. A J21, après vérification de l'installation des nématodes, la mise en lot a été réalisée. Un premier lot a reçu un granulé commercial sans TC auquel ont été ajoutées des pellicules de noisette (lot HG); un deuxième lot a reçu des granulés de sainfoin (SG); le troisième lot était le lot témoin (CG), recevant un granulé commercial libre de TC. Tous les lots étaient isoprotéiques et les agneaux des lots HG et SG ont été établis avec des niveaux équivalents de tanins. La période expérimentale de suivi s'est déroulée de J28 à J57, quand les agneaux ont été euthanasiés.

Des prélèvements individuels de fèces et de sang ont été réalisés de manière répétée pour mesurer les valeurs de coproscopies et d'hématocrite. De plus, une coproculture hebdomadaire a été réalisée par lot pour identifier et quantifiier la proportion des deux espèces de vers. A la suite des autopsies, les contenus digestifs du rumen, l'abomasum, l'intestin grêle et des fèces ont été prélevés pour analyses des populations parasitaires présentes (abomasum et intestin grêle) et mesures des tanins (Article 4).

Résultats et discussion

Les agneaux du lot consommant du sainfoin (SG) ont montré une diminution significative des comptages des œufs (FEC), ainsi que des valeurs plus élevées d'hématocrites ($P < 0.05$) par rapport aux animaux du lot témoin (CG) ou de ceux consommant des pellicules de noisette (HG). Pour le lot SG, les comptages des *H. contortus* ont montré une tendance à des nombres réduits ($P < 0.10$), tandis qu'aucune différence n'a été observée pour *T. colubriformis* entre les 3 lots. Aucune différence de fertilité des femelles d'*H.contortus* n'a été rapportée. Par contre, la fertilité des femelles des *T. colubriformis* du lot HG a montré une différence significative ($P< 0.05$) par rapport aux lots SG et CG.

Ces résultats suggèrent que le sainfoin, une ressource contenant des TCs avec un rapport PD/PC élevé a montré des résultats plus probants sur l'activité AH ainsi que pour améliorer *in vivo* la résilience de l'hôte par comparaison aux pellicules de noisettes qui présentent un rapport PD/PC bas alors que des niveaux similaires de TCs ont été recherchés. Ces résultats soutiennent l'idée que la qualité des TC influence aussi les effets AH en conditions expérimentales d'infestation *in vivo*.

Comparing the in *vivo* anthelmintic effects of two tannin-containing resources differing in tannin quality

Jessica Quijada[1,2], Elodie Gaudin[1,2,3], Ramzi El-Korso[1,2], Aina Ramsay[1], Irene Mueller-Harvey[4], Hervé Hoste*[1,2]

[1] INRA, UMR 1225, 23 Chemin des Capelles, Toulouse F-31076, France

[2] Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France

[3] MG 2 MIX, La Basse Haye, Châteaubourg 35220, France

[4] School of Agriculture, Policy and Development, University of Reading, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom

Abstract

The use of condensed tannin (CT)-containing plants seems a useful alternative approach to control gastrointestinal nematode infections (GIN) in ruminants. However, variation in anthelmintic (AH) results *in vitro* when using CT resources suggest that the quantity but also the quality (structure) of CT could play a role in the AH activity. Two resources with different structural features in regard to the prodelphinidin/procyanidin (PD/PC) ratio, namely sainfoin (*Onobrychis viciifolia*) and hazelnut skin (*Corylus avellana*), were offered to 4-month-old lambs (n= 27, nine per group) experimentally infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*, to evaluate the effect on parasite biology and host resilience. The group of lambs consuming sainfoin (SG) showed significantly lower faecal egg counts [(FEC) 1835.7±547.7 - 2800±802.7] and higher Packed Cell Volume [(PCV%) 30.3±1.1 – 35.7±1.6] ($p<0.05$) compared with the control group (CG) fed with tannin-free diet. Trends to lower total worm counts were observed for *H. contortus* in the SG ($P < 0.10$), whereas no differences were observed for *T. colubriformis*. Differences were observed for lower female worm fecundity for *T. colubriformis* ($P< 0.05$) in the lambs consuming hazelnut skin (HG), whereas no differences were observed for *H. contortus*. These results pointed out that sainfoin, a CT-containing resource with high PD/PC ratio, presented more consistent AH activity and improvement of host's resilience *in vivo* than hazelnut skin with low PD/PC ratio. These results support the hypothesis that both quantity and quality of CT influence the effects.

Keywords: condensed tannins*, Onobrychis viciifolia*, *Corylus avellana*, *Haemonchus contortus*, *Trichostrongylus colubriformis,* nematode, sheep

Introduction

Gastrointestinal nematode (GIN) infections remain one of the main constraints limiting the productivity and sustainability of grazing systems small ruminant production. For many years, the cornerstone to control worm populations has been based on repeated dosing with synthetic anthelmintic (AH) drugs. However, nowadays anthelmintic resistance has been described worldwide (Jackson et al., 2012). The use of bioactive plants with anthelmintic properties (nutraceuticals), especially condensed tannin-containing plants, has been proposed as an alternative or a complement to control nematode infections in sheep and goats (Hoste et al., 2015; Rochfort et al., 2008).

Condensed tannins (CT) are polyphenols, oligomers or polymers of flavan-3-ol units. They are produced as secondary metabolites in plants (PSM) and are considered as mechanism of plant's defense against predators, namely insects or herbivores (Salminen et al., 2011). Moreover, benefits of CT have been reported on animal health and production (Hoste et al., 2015; Mueller-Harvey, 2006; Rochfort et al., 2008). CT containing plants or forages have shown direct and/or indirect AH properties in *in vivo* studies with small ruminants. The AH effects include reducing GINs through decreasing either the 1) establishment of infective larvae (L3) or 2) faecal egg counts because of either reduction in worm burden (Diaz Lira et al., 2008; Mupeyo et al., 2011; Terrill et al., 2007) and/or lowering female fecundity (Manolaraki et al., 2010; Moreno-Gonzalo et al., 2014; Mupeyo et al., 2011; Paolini et al., 2003b). Moreover, 3) a delay or inhibition of egg development has been mentioned in a few studies (Mupeyo et al., 2011; Taylor et al., 2006; Valderrábano et al., 2010).

In addition, CT containing plants seem to improve the host's resilience. This is defined as the ability of animals to maintain production and preserve health under nematode challenge (Van Houtert and Sykes, 1996). Resilience is measured by either pathophysiological parameters [*e.g.* PCV (Paolini et al. 2003a)], clinical signs [e.g. diarrhea (Niezen et al., 1998), anemia (Paolini et al., 2005)] or mortality rates (Paolini et al., 2005).

Several results obtained *in vitro* have related the plants' CT content with the AH effect. Dose dependant effects have been observed on various key stages of GINs life cycle from ruminants for example: eggs (Moreno-Gonzalo et al., 2013b), larvae (Bahuaud et al., 2006; Novobilský et al., 2011; Novobilský et al., 2013) or adult worms (Hoste et al., 2009; Martínez-Ortíz-de-Montellano et al., 2013). Furthermore, such observations have been described *in vivo* in small ruminants, where the higher amount of CT-containing resource offered to the animals was related with lower GINs infection levels (Athanasiadou et al., 2001; Brunet et al., 2007; Terrill et al., 2009).

Nevertheless, variations in AH effects on GIN when CT-containing resources were studied led to hypothesize that the quality and/or structural features of CT could also play a role in the biological activity. Recent *in vitro* results pointed out that CT's monomer composition [*i.e.* prodelphinidin:procyanidin (PD/PC) ratio] and/or their size (*i.e.* mean degree of polymerization= mDP) are related to anthelmintic activity against either *Haemonchus contortus* (*i.e.* PD content and mDP) or *Trichostrongylus colubriformis* (*i.e.* PD content) (Quijada et al., 2015). Similar findings have been observed in nematodes of cattle (*Ostertagia ostertagi*, *Cooperia oncophora*) (Novobilský et al., 2013) or pigs (*Ascaris suum*) (Williams et al., 2014).

However, there is no information on such possible differences in *in vivo* studies. Several AH results have been shown with the CT containing temperate legume sainfoin (*Onobrychis viciifolia*) when offered to sheep or goats as fresh forage (Athanasiadou et al., 2005; Werne et al., 2013) hay (Paolini et al., 2005; Heckendorn et al., 2006), silage (Heckendorn et al., 2006), or pellets (Girard et al., 2013). On the other hand, endocarps of hazelnut (*Corylus avellana*) is a CT containing agro-industrial by-product and its consumption has been related to some AH properties. It can be offered directly or mixed with feed pellets to goats (Desrues et al., 2012) or sheep (Girard et al., 2013).

With regard to structural features of CT, those of sainfoin are mainly composed of prodelphinidin (PD), while procyanidins (PC) are the main component of hazelnut's endocarps or skin (Del Rio et al., 2011; Quijada et al., 2015; Williams et al., 2014). These two resources therefore offered posibilities to examine under *in vivo* conditions the effect of tannin's quality on the AH activity and consequently to better understand the structure/activity relationship of CT on nematode parasite infections.

Thus, the objectives of the current study were to compare the effects of sainfoin vs hazelnut skin on i) the biology of two nematodes species (from either the abomasum or the small intestine) in lambs, ii) the host's resilience.

Materials and Methods

1. Trial site

The experiment was carried out at ENVT (National Veterinary School of Toulouse), in the southwest of France (43°35′59″ N, 1°22′41″ E). The facilities hosting the animals and trial performance met the French ethical and welfare rules (agreement SSA N° 115 of December 15, 2014). The experimental site for each group, had concrete floors with separated boxes of approximate area of 12 m^2 each one. The animals had free access to water.

2. Animals

Twenty-seven 4-month-old lambs of Tarascon breed were used in the study. The lambs were raised under helminth-free conditions and found negative for strongyle nematode infections (by Mc Master technique according to Raynaud, 1970) before the start of the study. They were drenched with diclazuril (Vecoxan®, 2.5 mg/mL, Lilly-France, Neuilly-Sur-Seine, France), twice at three weeks interval, at the recommended dose of 1 mg/Kg of live weight to prevent coccidian infection. The animals were kept indoor.

3. Infective larvae

Susceptible strains of either *H. contortus* or *T. colubriformis* larvae were cultured from faeces of monospecifically infected donor sheep. Larvae were recovered through Baermann technique and then have been stored refrigerated (4 °C) for 1 month (*H. contortus)* or 4 months (*T. colubriformis)*.

4. Experimental design

On day 0 (D0), all lambs were orally infected with a single dose of 2000 L3 *H. contortus* and 2000 L3 *T. colubriformis*. They received *ad libitum* grass hay, mineral block and water, and a ration of commercial (tannin-free) pellets as well. On day 21 (D21) after parasite infection was confirmed by faecal examination, the animals were allocated into three groups of nine lambs, according to experimental diets [hazelnut skin= HG; sainfoin pellets= SG; control (tannin-free) pellets= CG]. The groups were balanced taking into account the sex, live bodyweight (mean 29.19 ± 2.71 Kg), PCV% (39.11 ± 2.38) and faecal egg counts (EPG= 1124.1 ± 370.8). From D24 to D28, a period was applied to let the lambs to adapt to the different diets. During the experiment (D28 – D57), the rations were adjusted once according to body weight (D34), to cover animal growing requirements. Therefore, from D37 to D44 a second adjustment period was applied for the three diets aiming to reach an optimal consumption level in the two CT-containing diets and to maintain isoproteic and isoenergetic levels in all groups.

All lambs were slaughtered by intravenous injection (3.6 g/lamb) of pentobarbital sodium (Doléthal®, 182.2 mg/mL, Vétoquinol S.A., Magny-Vernois, France) on day D57.

5. Tannin content in diets

Three methods were used to estimate the tannin content: the content of condensed tannins was estimated by acetone-butanol-HCl assay (Grabber et al., 2013). A standard *Onobrychis viciifolia* sample was used for all the measurements.

The total tannin content, was assessed according to the Folin-Ciocalteau method using tannic acid as standard (Makkar, 2000).

The polyphenols’ protein precipitation capacity was measured using the radial diffusion method (Hagerman and Butler, 1978), using tannic acid as standard.

6. Experimental feed

Three different diets were offered to the lambs according to the experimental group. The first group (HG) received plain commercial feed pellets (tannin free) + hazelnut endocarps; the second group (SG) was fed with sainfoin pellets; the third group (CG) was the control one and received plain commercial feed pellets (tannin free). All groups were offered grass hay *ad libitum*, mineral block and water. During the whole period of the study (*i.e.* 57 days), all groups received a fattening (total mixed) ration, which was isoproteic, isoenergetic and balanced for Ca, P and Ca: P ratio. Additionally, for the two CT-containing diets (*i.e.* SG and HG groups) an equal tannin concentration was offered based on measurements.

7. Measurements

7.1. Group refusals

Refusal were measured twice per week by weighing at 24-hour interval the difference in amount of concentrate feed [*i.e.* tannin-free pellets (CG) or mixed with hazelnut skin (HG) or sainfoin pellets (SG)] offered and the amount left.

7.2. Pathophysiological parameters

In order to estimate host resilience, individual blood samples were collected once weekly (from D0 until D55) by jugular venipuncture into heparinized tubes (Venosafe®, Terumo Europe, Belgium) to measure the packed cell volume (PCV) as an indicator of anemia according to the microhaematocrit method.

7.3. Parasitological parameters

Individual measurements were performed to characterize the effects of the different feed on worm biology by evaluating parasitological data either *in vivo* (faecal egg excretion) or during the *post mortem* procedures (worm counts and female worm fecundity).

Individual faecal samples were collected twice weekly directly from the rectum, from D21 until the end of the assay (D57) to determine faecal egg counts (FEC) using a modified McMaster technique (Raynaud, 1970). Faecal egg counts data were expressed as eggs per gram of faeces (EPG). Once per week from D21 until D55, larval cultures were prepared per group to determine the composition of GINs genera. The cultures were incubated at 23 °C for 10 days. Afterwards a Baermann technique was performed for larvae recovery. For genus identification, third stage larvae were transferred to a microscope slide, and 100 larvae per larval culture and date of sample were differentiated based on key of morphological identification (MAFF, 1986). The amount of EPG per parasite was estimated from the L3% of each species recovered and identified, as= [(EPG mean values per group) x (L3% per species)]/100.

At necropsy, the abomasa and the first 12 meters of small intestine were separated, tied, rapidly removed and then stored at − 20 °C for further adult worm counts. The worms were collected from the luminal contents of either the abomasum or small intestine. In addition, worms were recovered from the abomasal mucosa after a 4 h incubation in a pepsin solution at 37 °C. Worm counts were performed according to a 10% aliquot technique (MAFF, 1986). Morphological identification of worm stages, sex and species were conducted using standard recommendations (MAFF, 1986).

The fecundity of female worms was measured using 10 worms per lamb. For *T. colubriformis*, the eggs *in utero* were counted directly after clearing in 85% lactic acid solution. For *H. contortus*, the fecundity was determined using the method described by Kloosterman et al. (1978) with slight modifications. The worms were soaked in distilled water and then placed individually in microtubes with 1000 µL of 0.25% hypochloride solution. After 10 minutes at room temperature (24 - 26 °C) females worm disintegrated enabling the eggs counting using a 10% aliquot of the total volume. For both parasites, eggs counts were performed under a microscope at a 100x magnification.

8. Statistical Analyses

The data of FEC and the individual numbers of each nematode species as well as the total number of worms (*H. contortus* + *T. colubriformis*) recovered at necropsy were log 10(x+1) transformed prior to analysis. For the faecal egg excretion and the PCV values, comparisons were first performed using an analysis of variance on repeated measurements. Then, the comparison of results to the control values were carried out date by date relying on an one-way analysis of variance (ANOVA) completed by the post-hoc Bonferroni test. An ANOVA test with two factors (*i.e.* diet, individual) was applied to examine the differences in the fecundity of female worms (for both *H. contortus* and *T. colubriformis*) between the three experimental groups. All statistical analyses were performed using Systat® 9 software (SPSS Ltd).

Results

1. Tannin values in diets

In regard to CT content, hazelnut skin and sainfoin pellets showed a total content on CT of 5.1 and 6.5 g/100 g of dry weight (DW) respectively, whereas the control pellets were tannin-free as they did not show any reaction when the acetone-butanol-HCl assay was applied.

The total tannin contents were 22.1 and 4.2 (expressed as tannic acid equivalent) for hazelnut skin and sainfoin pellets, respectively.

When biological activity of protein precipitation capacity was analyzed for the CT resources, the CT values (expressed as tannic acid equivalent) were 15.29 for hazelnut skin and 2.73 for sainfoin pellets, respectively. There was no precipitation capacity observed with the control pellets.

Calculation of ratio of tannin content or biological activity gave hazelnut skin:sainfoin pellets ratio of 0.78 (CT content), 5.3 (total tannin content) and 5.6 (protein precipitation capacity), respectively.

On the basis of those results of tannin measurements, and because of the objective of our study, the quantity of sainfoin offered to lambs in the SG was ≈ 5-fold higher than the quantity of hazelnut skin added to control pellets in the HG. The aim was to make the diet of SG and HG groups similar in tannin content. Thereafter, the quantity of control pellets was adjusted in order to make, as far as possible the diets isoproteic.

Hence, as by acetone-butanol-HCl assay, different CT values were observed between the resources (6.5 vs 5.1 g CT/100 g DW in sainfoin and hazelnut skin, respectively) we decided to take into account the other two CT measurements assessed as well in order to estimate the amount of each resource to be offered to each experimental group, to ensure the isotannin level in both rations. Thus, according with the total of condensed tannin content through the radial diffusion, the results showed that hazelnut skin has around 5.5-fold more biological activity than sainfoin pellets. At the end of the trial, SG (n= 9 lambs) was offered with 3500 g of sainfoin pellets (*i.e.* each lamb was offered ≈ 16.2 g of total tannin and 1.4% of the ration), whereas HG (n= 9 lambs) was offered with 900 g of hazelnut skin mixed with tannin-free pellets. Therefore, the lambs in HG were offered ≈ 21 g of total tannin that was 1.7% of the ration per lamb.

2. Experimental feed, nutritional values

On the basis of feed nutritional analyses values (Table 1) and the tannin content in the hazelnut skin and sainfoin pellets, the different rations were calculated. Hence, three periods were considered for ration calculations during the trial. In the first trial period (D28 – D34), the ration composition regard to crude protein (CP) and CT in each experimental group (n= 9 lambs) were, respectively: in SG= 517.2 g, 126 g; in CG= 457.2 g, 0 g; and in HG= 53.0 g from hazelnut skin + 457.2 g from control pellets, for a total of CP of 510.2 g, CT value was 110g. At D34, the lambs were weighed in order to adjust the rations according with their growth. In the second period, from D37 to D44 an adjustment period was applied with the aim of reach the suitable consumption level. For the third period, from D45 to D57 the ration compositions (CP and CT) were, respectively: in SG= 603.4 g, 147 g; in CG= 579.1 g, 0 g; and in HG= 95.3 g from hazelnut skin + 472.4 g from control pellets, for a total of CP of 567.8 g, CT 198 g.

Therefore, in the first period the amounts of CP and CT offered to each lamb, according with the experimental group were, respectively: SG= 57.5 g, 14 g; in the CG= 50.8 g, 0 g; and HG, 56.2 g, 12.3 g. Finally, in the third period these values were: SG, 67.0 g, 16.3 g; CG, 64.3 g, 0 g and in the HG= 63.1 g, 22.1 g, respectively.

3. Group refusal values

The refusal (%) mean values per group through the trial period were: HG (hazelnut skin + control pellets) = 2.9 ± 1.3, SG (sainfoin pellets) = 0.3 ± 0.1 and CG (control pellets) = 0.6 ± 0.2. Along the whole experiment period, the highest refusal rank (0.56 – 9.59 %) were observed in the HG, showing differences with the other two groups ($P < 0.001$).

4. Pathophysiological variable

Overall, the PCV% mean values in SG were higher (30.3 ± 1.1 to 35.7 ± 1.6) than the two other groups since D28 (*i.e.* HG= 29 ± 1.4 to 31.8 ± 2.8, CG= 28.6 ± 1.3 to 31.3 ± 1.4) (Figure 1). However, the analysis of variance on repeated measures during the consumption of the full CT's ration (D28 – D55), revealed only a trend ($P < 0.086$) for higher PCV values in the SG compared with the two other groups. Indeed, from the date by date analyses, statistical differences were observed at D28 and D34 ($P < 0.05$) between SG and HG and CG groups.

5. Parasitology

5.1. Faecal Egg Counts (FEC)

Overall, the analyses of EPG based on the analysis of variance on repeated measures did not show any difference between groups. Then, the date by date analyses of Log(FEC+1) revealed some differences in the EPG mean per experimental group, altogether showed FEC increasing in the three groups through the experiment from D21 to D29 and no difference was observed (Figure 2). Once the lambs started consuming the experimental diets (D28), three periods of EPG excretion were recorded. First, from D34 until D41, statistical lower values were observed in SG compared with both CG and HG. Second, FEC did not show differences between groups from D44 through D51. Third, by the end of the experiment, differences in FEC were again recorded. Decreasing FEC were recorded in SG (D55, D57) compared with CG.

From D28 to D57, the range of mean FEC observed per group were: SG= 1835.7 ± 546.7 to 2800 ± 802.7, HG= 2077.8 ± 390.1 to 3461.1 ± 543.1 and for the CG= 2262.5 ± 198.3 to 3966.7 ± 520.2.

From D23 through D57 (necropsy day), the EPG mean values from SG were consistently lower than the values of the two other groups being statistically different by D36 ($P < 0.05$) and showing trends to be lower by D34, D41, D55 and D57 ($P < 0.10$). The range of

FEC decrease in SG was 14.6 – 40.5 %. The lambs in HG showed reduction in FEC (up to 21.1) compared with CG, however no significant differences were found.

5.2. Larval cultures

Altogether, *H. contortus* was the predominant species through the trial in all groups (Table 2). The mean values of L3 (%) for *H. contortus* were: HG= 93.3 ± 1.9, SG= 84.1 ± 5.8, CG= 86.2 ± 3.5 whereas for *T. colubriformis* they were: HG= 6.7 ± 1.9, SG= 15.9 ± 5.8, CG= 13.1 ± 3.5.

In addition, the EPG values per experimental group in regard of L3 recovered per species and per date, as presented in Figure 3 (A,B), showed lower EPG for *H. contortus* in SG compared with the other two groups, whereas lower EPG for *T. colubriformis* were observed in SG (from D34 to 48) and HG (from D28 to 48) compared with the control. On the basis of these values, the % of reduction in EPG mean values per parasite in the CT groups compared with the control group (Figure 4, A,B), overall EPG were reduced for *H. contortus* in the SG (4 - 51.2%), whereas for *T. colubriformis* in the HG (26.4 – 79.6%).

5.3. Adult worm counts

The total worm burden (*H. contortus* + *T. colubriformis*) between groups showed trends to be differents (P = 0.078), being higher in HG group. For *H. contortus*, total worm counts were lower in SG compared with HG and CG (Table 3), and trends were recorded (P = 0.106). In addition, trends for lower male count were observed (P = 0.079). Such differences were between the lower values in SG, compared with those of HG only, as was noted through the post-hoc Bonferroni (P= 0.082). Instead for *T. colubriformis*, similar worm burden were counted in all groups. No differences were observed for this species, neither in the total worm burden (P = 0.246) nor by sex (male, P = 0.384; female, P = 0.294).

The following sex ratio values (male:female) per species and per diet group were recorded, for *H. contortus* 0.65 (CG), 0.78 (HG) and 0.70 (SG), whereas for *T. colubriformis* 0.95 (CG), 0.85 (HG) and 0.87 (SG).

5.4. Female worm’s fecundity

For *T. colubriformis* female worms, the mean egg counts per worm were: HG= 24 ± 2.2, SG= 27 ± 2.1 and CG= 26 ± 2.0. These fecundity values showed differences between groups (P<0.001). The Bonferroni test revealed differences between the HG and the two other groups (P < 0.05).

However, for *H. contortus*, the mean egg counts per worm were: HG= 700 ± 122, SG= 742 ± 121 and CG= 682 ± 14. No differences in fecundity was observed between the three experimental groups (P > 0.05).

Discussion

During decades CT-containing resources have been underestimated because of possible negative effects on host metabolism and production (Mueller-Harvey, 2006). After several years studies on CT and the related resources, it has been shown that those with average values in tannin content < 50 g CT/Kg DM [measured by vanillin-HCl (Chiquette et al., 1989) or butanol-HCl (Terrill et al., 1992) methods], could be a profitable feed source and also contribute to animal health (Barry and McNabb, 1999; Hoste et al., 2015; Mueller-Harvey, 2006; Waghorn, 2008). In particular, temperate and tropical legumes have been shown to possess AH properties in ruminants (Alonso-Díaz et al., 2010; Hoste et al., 2012; Torres-Acosta et al., 2012; Waghorn, 2008;).

Nowadays, increasing interest exists in regard to agro-industrial by-products as cheap CT source for farmers as well as with an added value for industry. For instance, coffee (Vargas-Magaña et al., 2014), cocoa (Quijada et al., 2015; Vargas-Magaña et al., 2014), chestnut (Bahuaud et al. 2006) or hazelnut skin (Quijada et al., 2015) have shown AH effect *in vitro*.

Some *in vivo* studies have been conducted in small ruminants to evaluate AH effect with by-products (*i.e.* hazelnut skin) separately (Desrues et al. 2012) or in combination with sainfoin (Girard et al., 2013). So far, to the best of our knowledge, the current study is the first aiming at comparing the AH effect of by-products with CT forage (*i.e.* sainfoin) in the same experiment. The objective was to have differences in CT quality but no differences in CT quantity, in order to evaluate the actual effect of each CT's structural features (*i.e.* quality) on their AH activity. Therefore, the structural feature considered to choose both CT resources, and according with the findings of Quijada et al. (2015) was the PD/PC ratio, which was previously assessed (data not shown), and were 74.8/25.2 for sainfoin pellets and 27.9/72.1 for hazelnut skin. Instead, the mDP values in both resources were similar, 11.5 and 13.3 for sainfoin pellets and hazelnut skin, respectively.

Several methods/assays exist to evaluate the amount of tannin content in the different CT-containing resources. However, they measure different types of tannins and polyphenols, are referring to different standards and are providing different values (Mueller-Harvey, 2006). Thus, the recommended approach is to perform more than one assay, in order to have a better idea of the CT content in the resource/plant given (Salminen and Karonen, 2011). As mentioned before, values obtained from total tannin content and protein precipitation capacity (radial diffusion) were taken into account in order to calculate and ensure an isotannic value between the CT-containing diets (*i.e.* HG and SG). However, because the protein value in feed have been pointed out as the main nutritional component involved in the AH response, also an isoproteic value between the three groups was calculated (Houdijk, 2012).

One major restraint to consider when CT-containing forages or by-products are offered as feed, is the astringency which might affect its consumption. In the present study the CT-containing resources were well consumed, showing low refusal values for SG (< 1%). In the HG the mean refusal was 2.9%, being 100 g per lamb (D45 – D57) the higher amount offered. Nutritional and/or AH properties of sainfoin (*O. viciifolia*) given as forage, silage, hay or pellets has been evaluated in small ruminants, with promising results (Girard et al., 2013; Heckendorn et al., 2006; Paolini et al., 2005). Our results showed for the sainfoin pellets a similar consumption compared with the tannin-free control pellet. Additionally, a lower refusal of hazelnut skin was recorded compared with previous studies (Desrues et al., 2012; Girard et al., 2013), but still higher than the observed in the control group. It is worth to note that the percentage of refusal include hazelnut skin and control pellets.

The consumption of CT-containing resources in SG showed some positive effect on host resilience, measured by the PCV values. However, trends for higher PCV were observed in both CT groups compared with the control after full CT-rations consumption period (*i.e.* from D28 up to D55), contrary to described previously with regard to hazelnut skin consumption (Desrues et al., 2012; Girard et al., 2013). On the other hand, the PCV values in SG were higher than HG or CG during the five dates evaluated, showing significance in two of them. This improvement in hematocrit values has previously been reported in goats (Paolini et al., 2005) or sheep consuming sainfoin (Arroyo-Lopez et al., 2014; Azuhnwi et al., 2013). These effects on host resilience are important for survival and production (Urquhart et al., 1996).

In both groups consuming CT-containing resources reductions in FEC were observed. The FEC values observed in SG, were consistently lower than those from HG and CG during the trial period, with EPG reductions ranging from 13.6% (D55) to 40.5% (D36). The positive AH effect of decreasing in FEC has been observed in small ruminants fed with sainfoin regardless whether fresh, silage, hay or pellets (Athanasiadou et al., 2005; Girard et al. 2013; Heckendorn et al., 2006, 2007; Paolini et al., 2005; Werne et al., 2013). The pellets seem as a very convenient way to offer sainfoin to feed animals, with the advantages over the others due to facilitating management and stockage and long-lasting preservation.

In HG lower FEC compared with CG were recorded in five dates ranging from 3.76% (D48) until 21.1% (D44). However, these reductions were not significant. Conversely, Girard et al. (2013) observed trends in reduction of FEC in lambs when hazelnut skin was added to their sainfoin pellets ration and Desrues et al. (2012) recorded differences of FEC in goats fed with hazelnut skin as CT source. Such results might be related with the amount of hazelnut skin offered, which was higher in both aforementioned studies than in ours, for instance 500 g/goat/day (Desrues et al., 2012) or 300 g/lamb/day (Girard et al., 2013); however, the refusals were higher as well 30% (Desrues et al., 2012) and 68 – 82% (Girard et al., 2013), respectively. Another issue to take into account, is the host species (sheep/goat) and their different adaptations to cope with tannin diets, as increasing their tannin-binding salivary protein which could affect the tannin avalaibility for metabolism in digestive tract (Alonso-Díaz et al., 2012). Finally, in the results reported by Girard et al. (2013) might have occurred a synergistic or additive effect between the CT contained in the sainfoin pellets offered as a base diet plus the hazelnut skin added from D35 to D42.

Based on larval culture results, *H. contortus* was the predominant species through the trial in all the groups. This could be explained by *H. contortus* prolificacy (Neto-Padre et al., 2000), or because their eggs are better developing *in vitro* culture compared with *T. colubriformis* (Enermark, personal communication).

The positive effect on resilience and the AH effect observed on FEC reductions, and the lower total *H. contortus* observed in SG agree with several *in vivo* trials conducted with lambs experimentally infected with *H. contortus* and one intestinal species. In these trials, lambs after consumption of sainfoin, have also showed a diminution of *H. contortus* counts, but no effect on either *Cooperia curticei* (Heckendorn et al., 2007) or *T. colubriformis* (Arroyo-Lopez et al., 2014).

In our results, with the consumption of hazelnut skin no reduction in worm population was observed for *H. contortus* and *T. colubriformis*. Similar findings have been described in goats (Desrues et al., 2012) and sheep (Girard et al., 2013).

A previous study showed that, the consumption of hazelnut skin has reduced the fecundity of *H. contortus,* whereas lower no significant reduction in fecundity was observed for *T. colubriformis* (Desrues et al., 2012). Conversely, in our study only the fecundity of *T. colubriformis* showed differences in the HG compared with the other two groups. The lack of effect in the *H. contortus* female worm fecundity seems not surprising, as the effect of consumption of CT-resources on this parameter seems inconsistent. Thus sainfoin consumption has reduced the fecundity of *H. contortus* (Arroyo-Lopez et al., 2014; Manolaraki et al., 2010), *T. colubriformis* (Manolaraki et al., 2010) or no effect (Heckendorn et al., 2006; Ríos-de Alvarez et al., 2008).

In this study, an optimal level of protein was granted in each diet, and this is a critical nutrient in the immune response against parasites (Houdijk, 2012). Therefore, the three lamb groups seem managed to cope with their infections helped on the basis of a good nutritional status at a critical age when acquisition of immunity occurs. As stated

Athanasiadou et al. (2008) is the more convenient period to include PSM-containing resources, to profit the positive effect that they might produce with balanced diets on animal health. In fact, such additive effect could explain the positive results observed in the CT groups, on PCV and the diminution of FEC compared with the CG, although was only significant and consistent in the SG.

Conclusions

The lamb group consuming sainfoin pellets showed a clear and consistent reduction in FEC compared with the control group during the trial. Also significant response in their resilience, as measured in terms of anemia (PCV). The decrease FEC might be a consequence of lower total *H. contortus* counts in SG. In contrast, the lower fecundity of *T. colubriformis* female worms in HG was not related consistently with significant reduction of FEC. Some AH effect was observed in both CT groups, but consistent FEC reduction and improvement in host resilience (PCV) was only observed in SG. Finally, the AH effect observed in SG, seems to agree with *in vitro* results showing that CT with predominantly PD flavan-3-ol monomer composition (high PD/PC ratio) has a better AH activity, which is the case in *O. viciifolia*.

Acknowledgements

The financial support of the European Commission through the PITN-GA-2011-289377, "LegumePlus" project is sincerely acknowledged. The help from colleagues Mrs Marica Engström and Ms Liza Fonsou in collecting samples and feed the lambs is deeply grateful.

References

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Capetillo-Leal, C.M., 2012. Amino acid profile of the protein from whole saliva of goats and sheep and its interaction with tannic acid and tannins extracted from the fodder of tropical plants. *Small Ruminant Res.*, 103, 69–74.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? *Small Ruminant Res.*, 89, 164–173.

Arroyo-Lopez, C., Manolaraki, F., Saratsis, A., Saratsi, K., Stefanakis, A., Skampardonis, V., Voutzourakis, N., Hoste, H., Sotiraki, S., 2014. Anthelmintic effect of carob pods and sainfoin hay when fed to lambs after experimental trickle infections with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Parasite*, 21, 71.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.*, 99, 205–219.

Athanasiadou, S., Tzamaloukas, O., Kyriazakis, I., Jackson, F., Coop, R.L., 2005. Testing for direct anthelmintic effects of bioactive forages against *Trichostrongylus colubriformis* in grazing sheep. *Vet. Parasitol.*, 127, 233–243.

Athanasiadou, S., Houdijk, J. & Kyriazakis, I., 2008. Exploiting synergisms and interactions in the nutritional approaches to parasite control in sheep production systems. *Small Ruminant Res.*, 76, 2–11.

Azuhnwi, B.N., Hertzberg, H., Arrigo, Y., Gutzwiller, A., Hess, H.D., Mueller-Harvey, I., Torgerson, P.R., Kreuzer, M., Dohme-Meier, F., 2013. Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and faecal egg count in artificially infected lambs. *J. Anim. Sci.*, 91, 2343–2354.

Barry, T.N., McNabb, W.C., 1999. The implications of condensed tannins on the nutritive value of temperate forages fed to ruminants. *Br. J. Nutr*., 81, 263–272.

Bahuaud, D.; Martinez-Ortiz De Montellano, C.; Chauveau, S.; Prevot, F.; Torres-Acosta, F.; Fouraste, I.; Hoste, H. 2006. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology*, 132, 545-554.

Brunet, S., Aufrere, J., El-Babili, F., Fouraste, I., 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.

Chiquette, J. et al., 1989. Effect of tannin content in two isosynthetic strains of birdsfoot trefoil (*Lotus corniculutus* L.) on feed digestibility and rumen fluid composition in sheep little or no reduction in sheep. *Can. J. Anim. Sci.*, 1039(69), 1031–1039.

Del Rio, D., Calani, L., Dall'Asta, M., Brighenti, F., 2011. Polyphenolic composition of hazelnut skin. *J. Agric. Food Chem.*, 59, 9935–9941.

Desrues, O., Vargas-Magaña, J.J., Girard, M., Manolaraki, F., Pardo, E., Mathieu, C., Vilarem, G., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Jean, H., Hoste H., 2012. Can hazel-nut peels be used to control gastrointestinal nematodes in goats? XIth International Goat Conference, Las Canarias, Sept 2012.

Diaz Lira, C.M., Barry, T.N., Pomroy, W.E., McWilliam, E.L., Lopez-Villalobos, N., 2008. Willow (*Salix* spp.) fodder blocks for growth and sustainable management of internal parasites in grazing lambs. *Anim. Feed Sci. Technol*., 141, 61–81.

Girard M., Gaid S., Mathieu C., Vilarem G., Gerfault V., Routier M., Gombault P., Pardo E., Manolaraki F., Hoste H., 2013. Effects of different proportions of sainfoin pellets combined with hazel nut peels on infected lambs. 64th EAAP, Nantes, France, 26th -30th August, 2013.

Grabber, J.H., Zeller, W.E., Mueller-Harvey, I., 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in lotus species by the butanol-HCl-iron assay. *J. Agric. Food Chem*., 61, 2669–2678.

Hagerman, A.E., Butler, L.G., 1978. Protein precipitation method for the quantitative determination of tannins. *J. Agric. Food Chem*., 26, 809–812.

Heckendorn, F., Häring, D.A., Maurer, V., Senn, M., Hertzberg, H., 2007. Individual administration of three tanniferous forage plants to lambs artificially infected with *Haemonchus contortus* and *Cooperia curticei*. *Vet. Parasitol.*, 146, 123–34.

Heckendorn, F., Häring, D.A., Maurer, V., Zinsstag, J., Langhans, W., Hertzberg, H., 2006. Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* and *Cooperia curticei* in lambs. *Vet. Parasitol*., 142, 293–300.

Hoste, H., Brunet, S., Paolini, V., Bahuaud, D., Chaveau, S., Fouraste, I., Lefrileux, Y., 2009. Compared *in vitro* anthelmintic effects of eight tannin-rich plants browsed by goats in the southern part of France. In: Papachristou, T.G., Parissi, Z.M., Ben Salem, H., Morand-Fehr, P. (Eds.), Nutritional and Foraging Ecology of Sheep and Goats. Zaragoza: CIHEAM/FAO/NAGREF. CIHEAM/FAO/NAGREF, Zaragoza, pp. 431–436.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Vet. Parasitol*., 186, 18–27.

Hoste, H., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., Terrill, T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. *Vet. Parasitol.*, 212, 5–17.

Houdijk, J.G.M., 2012. Differential effects of protein and energy scarcity on resistance to nematode parasites. *Small Ruminant Res.*, 103, 41–49.

Jackson, F., Varady, M., Bartley, D.J., 2012. Managing anthelmintic resistance in goats-Can we learn lessons from sheep? *Small Ruminant Res.*, 103, 3–9.

Kloosterman, A., Albers, G.A., Van Den Brink, R. 1978. Genetic variation among calves in resistance to nematodes parasites. *Vet. Parasitol.*, 4, 353-368.

MAFF. 1986. Ministry of Agriculture, Fisheries, and Food. Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationery Office, London, UK.

Makkar, H.P.S., 2000. Quantification of tannins in tree foliage, A laboratory Manual for the FAO/IAEA. Vienna.

Manolaraki, F., Sotiraki, S., Stefanakis, A., Skampardonis, V., Volanis, M., Hoste, H. 2010. Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. *Parasitology*, 137, 685-696.

Martínez-Ortíz-de-Montellano, C., Arroyo-López, C., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Hoste, H., 2013. Scanning electron microscopy of *Haemonchus contortus* exposed to tannin-rich plants under *in vivo* and *in vitro* conditions. *Exp. Parasitol.*, 133, 281–6.

Moreno-Gonzalo, J., Manolaraki, F., Frutos, P., Hervás, G., Celaya, R., Osoro, K., Ortega-Mora, L.M., Hoste, H., Ferre, I., 2013. *In vitro* effect of heather (Ericaceae) extracts on different development stages of *Teladorsagia circumcincta* and *Haemonchus contortus*. *Vet. Parasitol.*, 197, 235–243.

Moreno-Gonzalo, J., Osoro, K., García, U., Frutos, P., Celaya, R., Ferreira, L.M.M., Ortega-Mora, L.M., Ferre, I., 2014. Anthelmintic effect of heather in goats experimentally infected with *Trichostrongylus colubriformis*. *Parasitol. Res.*, 113, 693–699.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.*, 86, 2010–2037.

Mupeyo, B., Barry, T.N., Pomroy, W.E., Ramírez-Restrepo, C. a., López-Villalobos, N., Pernthaner, a., 2011. Effects of feeding willow (*Salix* spp.) upon death of established parasites and parasite fecundity. *Anim. Feed Sci. Technol.*, 164, 8–20.

Neto-Padre, L., Afonso-Roque, M.M., Fazendeiro, I., Refega, S., Cabaret, J., 2000. Digestive-tract strongyle faecal egg counts in cattle, sheep and goats of Sao Tomé Island in relation to local climate, season and breeding management. *Rev. élev. Méd. vet. Pays trop.*, 53, 263–266.

Niezen, J.H., Robertson, H.A., Waghorn, G., Charleston, W.A., 1998. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Vet. Parasitol.*, 80, 15–27.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. *Vet. Parasitol.*, 182, 213–20.

Novobilský, A., Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Enemark, H.L., Mueller-Harvey, I., Thamsborg, S.M., 2013. *In vitro* effects of extracts and purified tannins of sainfoin (*Onobrychis viciifolia*) against two cattle nematodes. *Vet. Parasitol*., 196, 532–7.

Paolini, V., Bergeaud, J.P., Grisez, C., Prevot, F., Dorchies, P., Hoste, H., 2003a. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.*, 113, 253–261.

Paolini, V., De La Farge, F., Prevot, F., Dorchies, P., Hoste, H., 2005. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. *Vet. Parasitol.*, 127, 277–283.

Paolini, V., Frayssines, A., Farge, F., Dorchies, P., 2003b. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Vet. Res.*, 331-339.

Quijada, J., Fryganas, C., Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., Hoste, H., 2015. Anthelmintic activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from small ruminants are influenced by structural features of condensed tannins. *J. Agric. Food Chem*., 63, 6346–6354.

Raynaud, J.P. 1970. Etude de l'efficacité d'une technique de coproscopies quantitative pour le diagnostic de routine et le contrôle des infestations parasitaires des bovins, ovins, équins et porcins. *Ann. Parasitol.*, 45, 321-342.

Ríos-de Alvarez, L., Greer, A.W., Jackson, F., Athanasiadou, S., Kyriazakis, I., Huntley, J.F., 2008. The effect of dietary sainfoin (*Onobrychis viciifolia*) on local cellular responses to *Trichostrongylus colubriformis* in sheep. *Parasitology,* 135, 1117–1124.

Rochfort, S., Parker, A.J., Dunshea, F.R., 2008. Plant bioactives for ruminant health and productivity. *Phytochemistry*, 69, 299–322.

Salminen, J.P., Karonen, M., 2011. Chemical ecology of tannins and other phenolics: We need a change in approach. *Funct. Ecol.*, 25, 325–338.

Salminen, J.P., Karonen, M., Sinkkonen, J., 2011. Chemical ecology of tannins: Recent developments in tannin chemistry reveal new structures and structure-activity patterns. *Chem. - A Eur. J.*, 17, 2806–2816.

Taylor, P., Waghorn, T.S., Molan, A.L., 2006. *In vivo* anthelmintic activity of *Dorycnium rectum* and grape seed extract against *Ostertagia* (*Teladorsagia*) circumcincta and *Trichostrongylus colubriformis* in sheep. *N. Z. Vet. J.*, 54, 21–27.

Terrill, T.H., Rowan, A.M., Douglas, G.B., Barry, T.N. 1992. Determination of extractable and bound condensed tannin concentration in forage plants, protein concentrate meals and cereal grains. *J. Sci. Food Agric.*, 58, 321-329.

Terrill, T.H., Dykes, G.S., Shaik, S. A., Miller, J.E., Kouakou, B., Kannan, G., Burke, J.M., Mosjidis, J. A., 2009. Efficacy of sericea lespedeza hay as a natural dewormer in goats: dose titration study. *Vet. Parasitol.*, 163, 52–6.

Terrill, T.H., Mosjidis, J. a, Moore, D. A., Shaik, S. A., Miller, J.E., Burke, J.M., Muir, J.P., Wolfe, R., 2007. Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. *Vet. Parasitol.*, 146, 117–22.

Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Hoste, H., Aguilar-Caballero, A. J., Cámara-Sarmiento, R., Alonso-Díaz, M. A. 2012. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. *Small Ruminant Res.*, 103, 28–40.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W. 1996. Veterinary Parasitology, 2nd Ed, Blackwell Science. Oxford, UK; 307 p.

Valderrábano, J., Calvete, C., Uriarte, J., 2010. Effect of feeding bioactive forages on infection and subsequent development of *Haemonchus contortus* in lamb faeces. *Vet. Parasitol.*, 172, 89–94.

Van Houtert, M.F.J., Sykes, A.R., 1996. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *Int. J. Parasitol.*, 26, 1151–1167.

Vargas-Magaña, J.J., Torres-Acosta, J.F.J., Aguilar-Caballero, A. J., Sandoval-Castro, C. A., Hoste, H., Chan-Pérez, J.I., 2014. Anthelmintic activity of acetone–water extracts against *Haemonchus contortus* eggs: Interactions between tannins and other plant secondary compounds. *Vet. Parasitol*., 206, 322–327.

Waghorn, G., 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. *Anim. Feed Sci. Technol.*, 147, 116–139.

Werne, S., Perler, E., Maurer, V., Probst, J.K., Hoste, H., Drewek, A., Heckendorn, F., 2013. Effect of sainfoin (*Onobrychis viciifolia*) and faba bean (*Vicia faba*) on the periparturient rise in ewes infected with gastrointestinal nematodes. *Small Ruminant Res.*, 113, 454–460.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One 9*, DOI: 10.1371/journal.pone.0097053

Table 1. Feed nutritional analyses results for each experimental group (hazelnut skin was added to control pellets for the HG group ration). (ADF, acid detergent fiber; ADL, acid detergent lignin; UFL, unit of energy, unit of forage for milk production; UFV, unit of energy, unit of forage for meat production; PDIA, dietary protein truly digestible in the small intestine; PDIE, true protein absorbable in the small intestine, when energy is limiting in the rumen; PDIN, true protein absorbable in the small intestine, when nitrogen is limiting in the rumen; NP, not performed)

		hazelnut skin (HG)		sainfoin pellets (SG)		control pellets (CG)	
		/net	/DM	/net	/DM	/net	/DM
Dry matter (DM)	(g/kg)	897.8	1000	891.7	1000	885.6	1000
composition according with Weende's schema							
crude protein	(g/kg)	105.9	117.9	172.4	193.3	152.4	172.1
crude fiber	(g/kg)	134.7	150.0	198.0	222.1	76.0	85.8
crude fat	(g/kg)	266.9	297.3	30.9	34.7	28.7	32.4
ash	(g/kg)	41.0	45.6	71.3	80.0	69.8	78.8
N-free extract	(g/kg)	349.4	389.1	419.1	470.0	558.7	630.8
Van Soest's fiber							
ADF	(g/kg)	NP		NP		90.8	102.6
ADL	(g/kg)	NP		NP		29.9	33.8
nutritional values							
UFL	(/kg)	NP		0.69	0.77	0.84	0.95
UFV	(/kg)	NP		0.62	0.69	0.80	0.9
PDIA	(g/kg)	NP		38	43	87	98
PDIE	(g/kg)	NP		84	94	115	130
PDIN	(g/kg)	NP		99	111	119	134

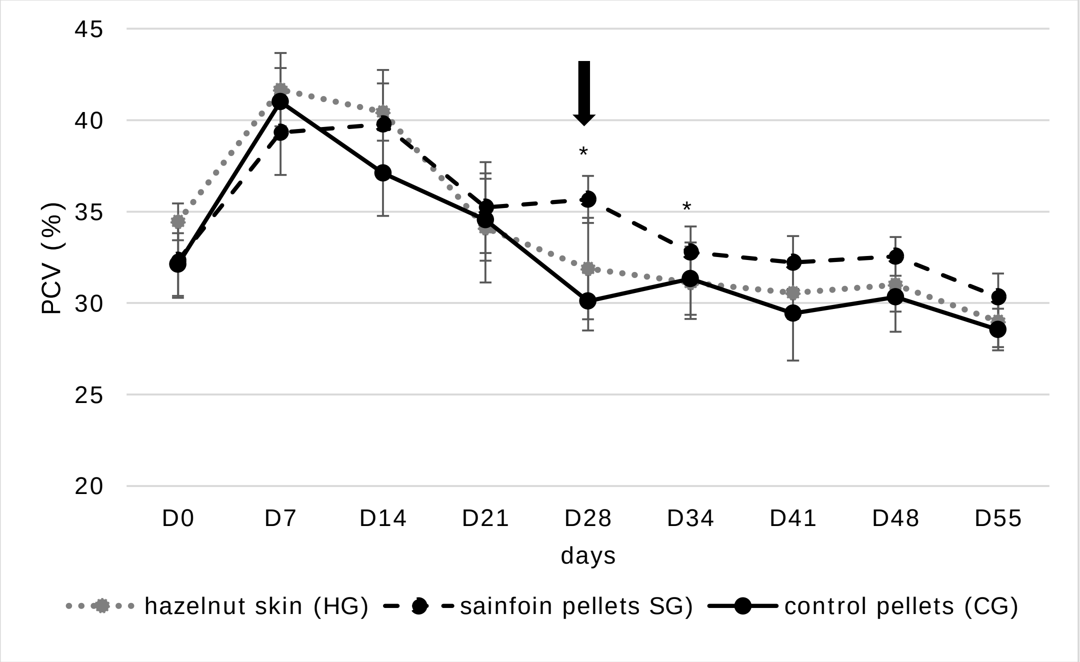


Figure 1. Mean values of Packed cell volume (%) in each lambs' group. (* Date of the survey when significant differences ($P < 0.05$) were observed between the SG and the two other groups). The arrow shows the beginning of full diet consumption

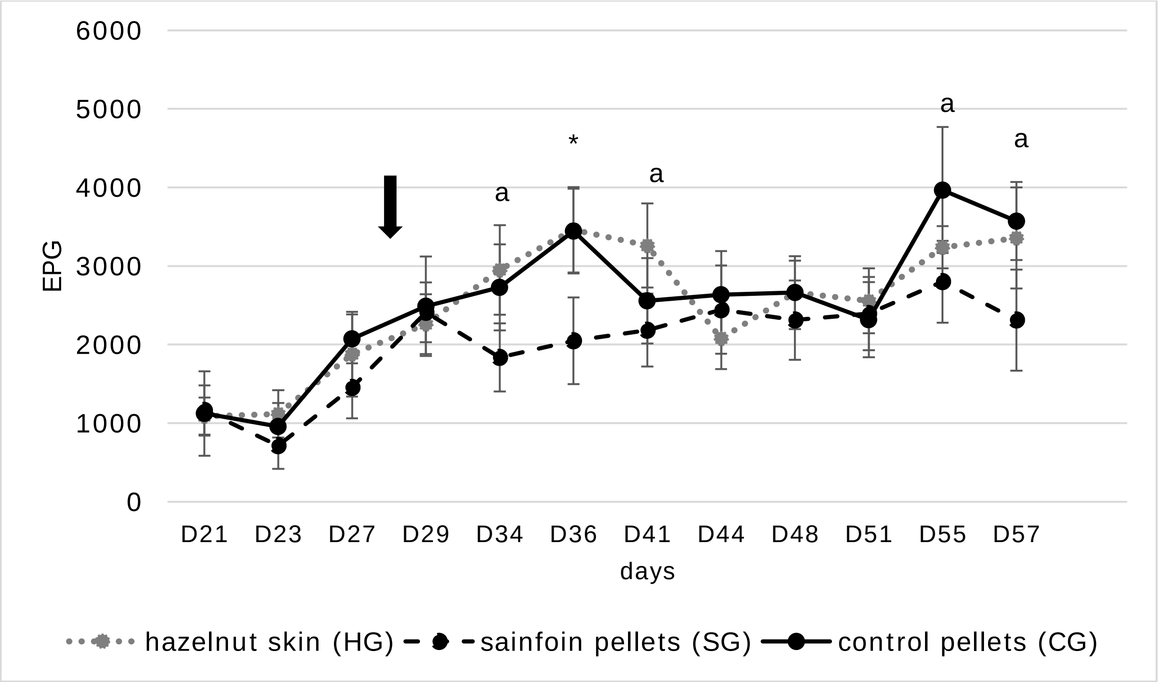
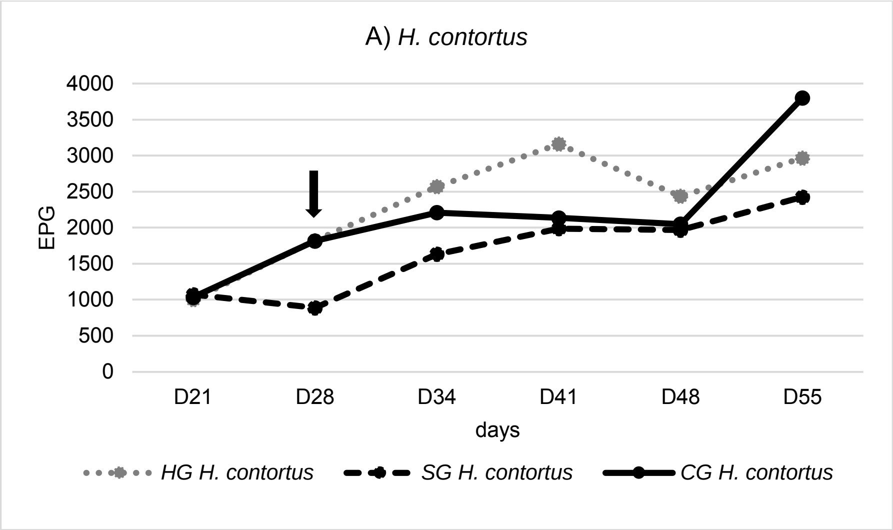


Figure 2. Fecal egg counts (FEC) mean values in each lambs group. (Date of the survey when significant differences [* $P < 0.05$) or trend (^{a} $P < 0.10$) were observed between the SG vs. the two other groups]. The arrow shows the beginning of full diet consumption



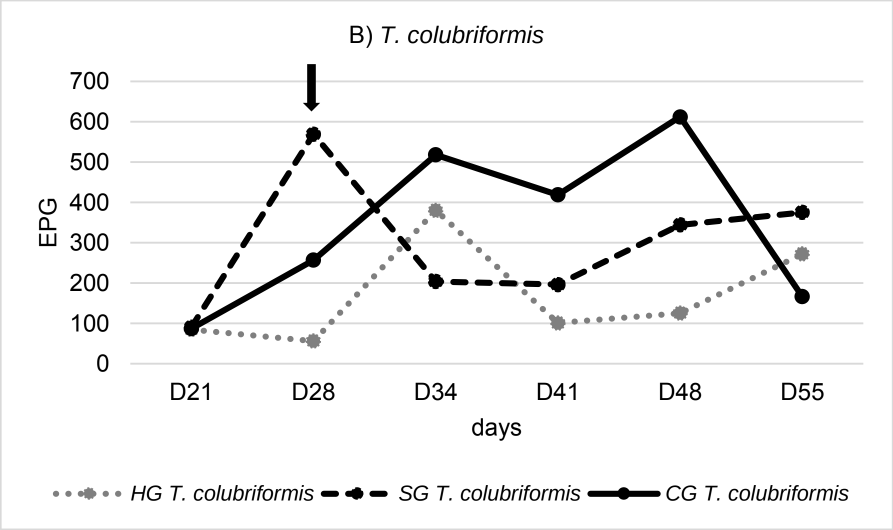


Figure 3. EPG values per species according with L3 (%) recovered from larval culture in each experimental group. A) *H. contortus,* B) *T. colubriformis.* The arrow shows the beginning of full diet consumption

Table 2. Third stage larvae (L3) (%) of *H. contortus* and *T. colubriformis* recovered from larvae culture per species in lambs experimentally infected and fed an isoproteic and isoenergetic diet of hazelnut skin (HG), sainfoin pellets (SG) and control pellets (CG), respectively

group	*H. contortus* (% of L3)							*T. colubriformis* (% of L3)						
	D21	D28	D34	D41	D48	D55	$\overline{X}$ value	D21	D28	D34	D41	D48	D55	$\overline{X}$ value
CG	92.2	87.6	81.0	83.6	77.0	95.8	86.2 ± 3.5	7.8	12.4	19.0	16.4	23.0	4.2	13.1 ± 3.5
HG	92.2	97.0	87.1	96.9	95.1	91.6	93.3 ± 1.9	7.8	3.0	12.9	3.1	4.9	8.4	6.7 ± 1.9
SG	92.2	60.9	88.9	91.0	85.1	86.6	84.1 ± 5.8	7.8	39.1	11.1	9.0	14.9	13.4	15.9 ± 5.8

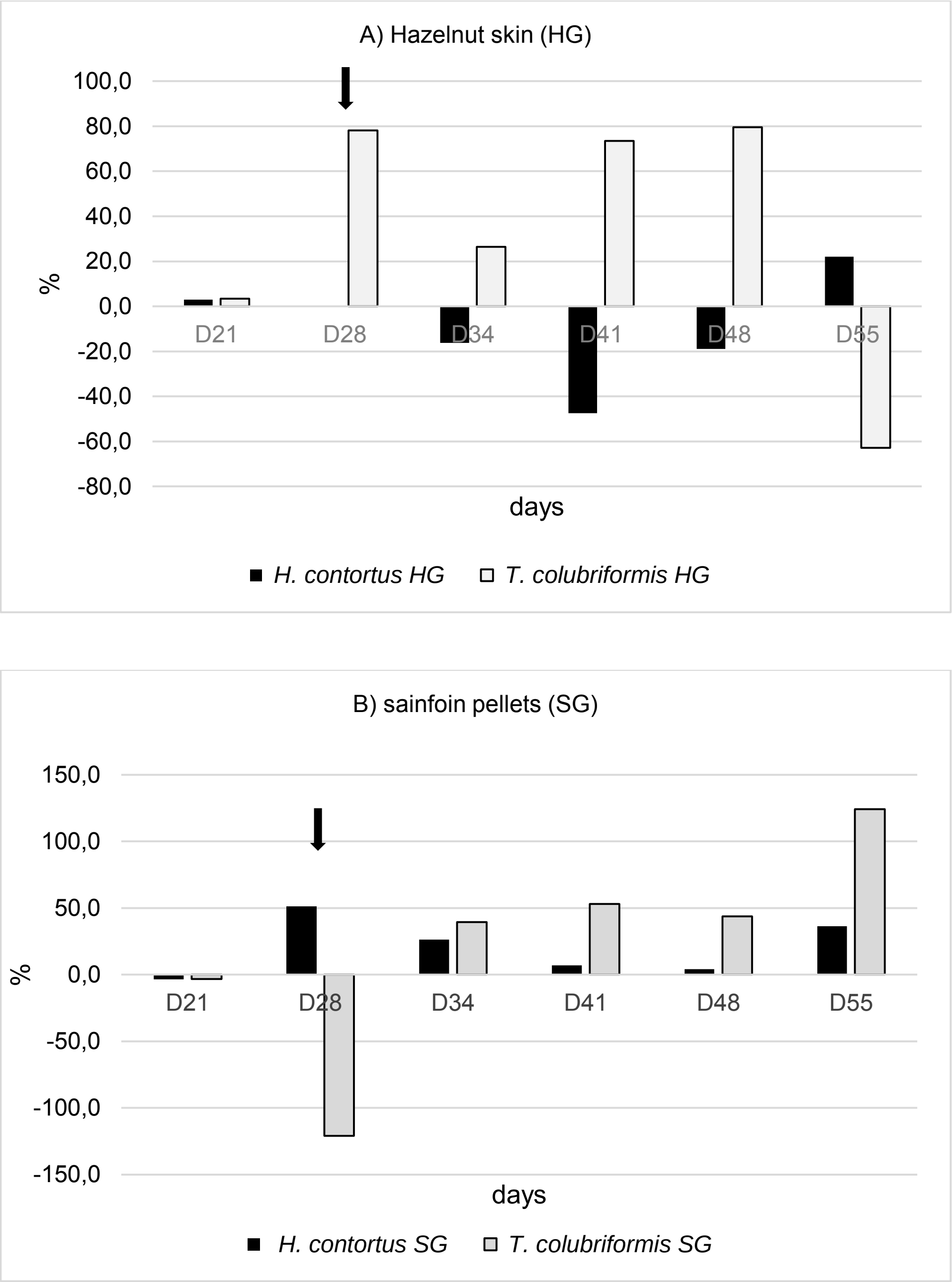


Figure 4. Percentage of reduction in EPG mean values per parasite in lambs group fed with condensed tannin-containing resources, compared with the control pellets group (CG). A) HG= hazelnut skin (+ tannin-free pellets) group; B) SG= sainfoin pellets group. (The arrow shows the beginning of full diet consumption)

Table 3. Mean adult worm counts (by sex and total) per parasite species, and total worm burden in each experimental group [HG= group fed with hazelnut skin as CT source (plus tannin-free pellet), SG= group fed with sainfoin pellets, CG= control group]. ^{a} trends (P<0.10) in differences were observed between the HG and the SG groups.

group	*H. contortus*			*T. colubriformis*			Total worm burden *(H. contortus + T. colubriformis)*
	♀	♂	Total	♀	♂	Total	
HG	244 ± 53.6	189 ± 36.2	433 ±85.8	357 ± 54.6	304 ± 39	661 ± 83.9	1095 ± 140.2^{a}
SG	168 ± 40.9	118 ± 26.0^{a}	286 ± 64.9	323 ±46.2	283 ± 26.2	606 ± 62.5	892 ± 94.1
CG	207 ± 41.1	135 ± 30.3	342 ± 70.4	273 ± 69.2	260 ± 52.8	533 ±109.8	875 ± 143.5

CHAPITRE 4

CHAPITRE 4 : Etude *in vivo* des Relations Structure/Activité des Tanins Condensés et de leur Disponibilité au niveau du Contenu Digestif en relation avec la Quantité et la Qualité

ARTICLE 4 : Contenu et réactivités des tanins condensés dans le tube digestif des moutons

QUIJADA J., DRAKE, C., GAUDIN, E. EL-KORSO, R., HOSTE, H. et MUELLER-HARVEY, I.

Manuscrit en préparation

RESUME – ARTICLE 4

Introduction

Les tannins sont un groupe de molécules hydrosolubles. Ce sont des métabolites secondaires des plantes qui ont de nombreuses propriétés bénéfiques (Salminen et al., 2011). Les tannins condensés (TC) ou proanthocyanidins (PA) sont principalement des polymères constitués d'unités flavan-3-ols (epi)catéchine et (epi)gallocatéchine (*i.e.* PD: prodelphinidine ou PC: procyanidine) (Patra et Saxena, 2011).

Des études *in vitro* et *in vivo* sur des plantes contenant des TCs ont montré qu'ils étaient dotés d'activité anthelminthique (AH) contre les nématodes gastro-intestinaux (GINs). A coté d'effets liés à la concentration, certaines études ont suggéré une relation entre la structure des TCs et leurs propriétés AH. Par ailleurs, des différences de sensibilité ont été notées en fonction de l'espèce de ver ou selon l'organe du tube digestif parasité. Par conséquent, plusieurs problématiques restent à éclaircir poru mieux comprendreles effets anthelminthiques des TCs. Deux hypothèses peuvent être proposées pour expliquer les différences de sensibilité des nematodes soit une différence de réponse spécifique intrinsèque ou ii) soit une différence d'exposition des vers aux TCs en fonction de leur biodisponibilité le long du tube digestif.

L'étude présente a eu pour but d'évaluer la biodisponibilité des TCs lors du transit le long du tube digestif de mouton en mesurant les variations de quantité (concentration) et de qualité (structure) afin de mieux appréhender si de telles variations peuvent expliquer les variations d'activité AH des TCs *in vivo*

Matériel et Méthodes

A l'autopsie, 200 mL des contenus digestifs ont été prélevés sur cinq agneaux par groupe expérimental [granulés de sainfoin (SG), pellicule de noisette (HG), témoin (CG)] directement dans le rumen, l'abomasum et intestin grêle ainsi que des fèces du rectum. Les échantillons ont été congelés, lyophilisés, broyés (< 1 mm) et conservés à − 20 °C en attente d'analyse.

Les analyses de tannins ont été faites d'abord par mesure colorimétrique (méthode de Graber à l'acétone-HCl-butanol) sur 10 mg d'échantillon de l'alimentation (granulés de sainfoin, granulés témoin ou pellicule de noisette) ou sur des échantillons de contenu digestifs ou de fèces lyophilisés. Les concentrations en CT sont exprimées par g/100 g de matière sèche (MS). Par ailleurs, les tannins condensés ont aussi été dosés par la méthode de dégradation chimique par Thiolyse en présence de benzyl mercaptan. Une solution de d'acide formique à 1 % (9 mL) est ajoutée afin de d'arrêter la réaction et les échantillons sont analysés par LC-MS (280 nm).

Résultats et Discussions

Des pertes importantes de CTs ont été observés pour les deux groupes expérimentaux d'animaux (sainfoin et noisette) dans les divers compartiments digestif (rumen, estomac, intestin grêle) quelque soit la méthode d'analyse des CTs par rapport à l'aliment. A l'inverse, pour les paramètres qualitatifs (de structure), peu de différences dans les ratios PD/PC ; *cis* / *trans* et les mDP ont été constatées dans les échantillons de digesta et de feces. La composition en monomères flavanols semble également être majoritairement préservée pour les deux groupes. Ces résultats suggèrent que les CTs du sainfoin ou de la noisette ne sont pas inertes le long du tube digestif mais subissent différentes réactions potentielles, notamment en réagissant avec d'autres molécules.

En conclusion, les résultats ont montré que les CTs présents dans les digesta et les fèces des moutons ont subit des variations de concentrations durant leur passage le long du tube digestif. Cette importante disparition des CTs pourrait être liée à leur dégradation, leur absorption ou à leur complexation avec des macromolécules. Pour leur part, les paramètres de structure des CTs semblent globalement préservés de même que la composition en monomères flavanols. Les CTs présents dans les pellicules de noisette sont apparus plus réactifs et ceux du sainfoin plus stables ce qui affecte certainement leur biodisponibilité et leur activité AH.

Tannin content and reactivities in the digestive tract of sheep

Jessica Quijada[1,2], Christopher Drake[3], Elodie Gaudin[1,2,4], Ramzi El-Korso[1,2], Hervé Hoste[1,2], Irene Mueller-Harvey[4]

[1] INRA, UMR 1225, 23 Chemin des Capelles, Toulouse F-31076, France

[2] Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France

[3] School of Agriculture, Policy and Development, University of Reading, 1 Earley Gate, P.O. Box 236, Reading RG6 6AT, United Kingdom

[4] MG 2 MIX. La Basse Haye, Châteaubourg 35220, France.

Abstract

Evidence of anthelmintic (AH) effects of condensed tannins (CT) against gastrointestinal nematode (GIN) infections in small ruminants have been described *in vitro* and *in vivo*. However, variations in AH results depending on the nematode species led to question whether this is due to differences in parasite susceptibility and/or variation in exposure to CT quantity and/or features. The present study aimed at evaluating the variation of CTs during their transit through the digestive tract in regard of quantity (concentration) and/or quality (structure). Digesta and faeces samples of experimentally infected (*Haemonchus contortus*, *Trichostrongylus colubriformis*) lambs (n= 15) consuming contrasting CT-resources [*i.e.* with different PD/PC, namely sainfoin pellets (SG) vs hazelnut skin (HG); control tannin-free pellets (CG)] were analyzed for CT content and structure. The CT content values observed for both SG and HG digesta and faeces were lower than those present in the feed, and showed similar patterns by compartment, irrespective of the CT resource. The disappearance of CT along the gastrointestinal tract seems related to conformational changes and their complexation with other molecules present in digesta. However, due to the large CT disappearance, the hypotheses of their degradation and/or absorption may not be discarded. The structure features (quality) seemed preserved in digesta in both diets. Further studies are needed to reveal the actual impact of these CT reactivies in the AH activity.

Keywords: condensed tannins, nematode, *Onobrychis viciifolia*, *Corylus avellana*, flavan-3-ols, acetone-HCl-butanol, thiolysis

Introduction

Tannins are a large group of water-soluble polyphenols. They are plant secondary metabolites (PSM) with various beneficial properties for plants (Salminen et al., 2011). Tannins are usually classified in: hydrolysable tannins (HT) and condensed tannins (CT). The HTs are complex molecules with a polyol as a central core (*e.g.* glucose, glucitol, quinic acids, quercitol, shikimic acid) which is partially or totally esterified with a phenolic group (*i.e.* gallic acid). Meanwhile, CTs or proanthocyanidins (PA) are mainly polymers of the flavan-3-ol (epi)catechin and (epi)gallocatechin units (*i.e.* PD, prodelphinidin or PC, procyanidin monomers) including their gallated forms, which are linked by C4−C8 and C4−C6 interflavonoid linkages (Hagerman, 2012; Patra and Saxena, 2011) .

It is assumed that CT main properties explaining their positive effect in health and animal production, are associated with their protein binding capacity and antioxidant activity (Luciano et al., 2011; Mueller-Harvey, 2006). The first property, has been shown to be responsible for improvement of protein utilization and therefore, animal production (Buccioni et al., 2015; Patra and Saxena, 2011), decreasing methanogenesis in rumen of ruminants (Bhatta et al., 2015; Liu et al., 2011) and anthelmintic activity (Hoste et al., 2015; Zhong et al., 2014).

Our interest is focused on anthelmintic (AH) activity of CT, against gastrointestinal nematodes (GIN) both *in vitro* or *in vivo* (Hoste et al., 2012; Hoste et al., 2015). Some *in vitro* and *in vivo* results support a dose-dependent AH response (Athanasiadou et al., 2001; Brunet and Hoste, 2006; Brunet et al. 2007; Burke et al., 2011; Moreno-Gonzalo et al., 2013a). However, the quantity of CT is not always related to AH activity (Naumann et al., 2014; Novobilský et al., 2013). Indeed, evidence recently have been reported of structure/activity relationship in AH activity against parasites from either cattle (Novobilský et al., 2011), small ruminant (Quijada et al., 2015) or pigs (Williams et al., 2014), suggesting that the polymer size (mDP, mean degree of polymerization) and monomer structure or quality (*i.e.* PD/PC ratio) of CT can modulate their AH effect. Additionally, Quijada et al. (2015) observed *in vitro* that parasite species did show differences in susceptibility to CT in relation with the structure. Namely, the AH activity against *Haemonchus contortus* (abomasal species) seems related to both mDP and PD/PC ratio features, whereas for *Trichostrongylus colubriformis* (a small-intestinal worm) PD/PC ratio seems to be the main factor.

On the other hand, evidence from *in vitro* and *in vivo* studies, have shown differences in AH effects of CT resources against different GINs (Hoste et al. in press), in particular between species inhabiting the abomasum or the small intestine. For instance in experimentally infected sheep, a strong AH effect (reduction of adult worm burden and female fecundity) of quebracho's CT were observed on intestinal species (*Nematodirus battus* and *T. colubriformis*) whereas no AH effect was observed for abomasal species (*Teladorsagia circumcincta* and *H. contortus*) (Athanasiadou et al., 2001). However, in goats, the same CT resource provoked reduction in worm burden for *T. colubriformis,* lower fecundity for *H. contortus* but no changes for *T. circumcincta* (Paolini et al., 2003a; Paolini et al., 2003b). Similar variations with regard to GINs species have been observed *in vitro*. Moreno-Gonzalo et al. (2013a; 2013b) evaluated the AH effect of heather (Ericaceae) extracts on the exsheathment process of *T. circumcincta, H. contortus* and *T. colubriformis* infective larvae (*i.e.* L3, using LEIA). The EC50 results indicated a higher susceptibility for *T. colubriformis* than for the abomasal species *H. contortus* or *T. circumcincta.* However, when CT purified fractions from 15 different CT-resources were evaluated *in vitro* by applying LEIA, overall lower EC50 were recorded for *H. contortus* (more susceptible) compared with *T. colubriformis* (Quijada et al., 2015). Consequently, several issues remain to be addressed related to the AH effect of CTs. To explain such variations in AH activity against GINs, two hypotheses might be proposed, whether i) it is a specific species response or ii) differences in CT bioavailability along the digestive tract (Desrues et al., 2015; Terrill et al., 1994).

On the basis of the complexity of CT structure/activity relationship and their metabolism/complexation in the digestive process, few studies have been performed on the CT content and their reactivities along the gut in ruminants. (Desrues et al., 2015; Perez-Maldonado and Norton, 1996; Terrill et al., 1994). Therefore, due to this lack of information in regard of CT and their relation with effects on GIN infections, the present study was conducted aiming at evaluating the possible variations of CTs (*i.e.* PD vs PC mainly polymer structure, namely sainfoin vs hazelnut skin) in their transit through the digestive tract of sheep in regard of: i) quantity (concentration), and/or ii) quality (structure); in order to assess if such variations can be related to the *in vivo* AH activity of these CT resources. For this, we harnessed animals experimentally infected with *H. contortus* and *T. colubriformis*, set up in three experimental groups receiving hazelnut skin, sainfoin pellets or a control diet (Article 3).

Materials and Methods

A detailed description of the trial site, animals, experimental feed, and experimental design has been included in the "Materials and Methods" section of the Article 3. We will focus here on specific measurements required to address the specific objectives mentioned above.

1. Preparation of digesta and faecal samples

At necropsy, individual samples of digesta were collected from five lambs (out of nine) per experimental group (*i.e.* sainfoin pellet, SG; hazelnut skin, HG; control, CG). Namely, around 200 mL of whole digestive content (digesta) were collected directly from each organ rumen, abomasum or small intestine (ileum). Additionally, individual faeces samples were taken from rectum. Each sort of sample was poured individually into a 500 mL volume foil container and was stored, frozen, at -20 °C.

The frozen samples (*e.g.* digesta or faeces) were put into the freeze-dryer (Cryotec, MUT PCCPLS1.5 001, France) and freeze-dried at -40 °C (-0.5 °C/min) during 2 hours. The freeze-drying process was carried-out in two phases. Firstly, samples underwent a progressive freeze-drying process at the following temperatures, decreasing temperature speed and pressures: -30 °C (0.1 °C/min, 0.1 mbar), then at -10 °C (0.2 °C/min, 0.3 mbar during 19 h 45′), finally -5 °C (0.2 °C/min, 0.15 mbar) until reaching -2 °C. The second phase started once the samples had reached a temperature of -2 °C. They were kept at 20 °C with a pressure of 0.05 mbar during 15 to 20 hours until no water was left in the samples. Afterwards, the freeze-dried samples of digesta or faeces were ground in a Retsch impeller SM1 cutting mill (Haan, Germany) to pass <1 mm, and then kept frozen at -20 °C until being analysed for CT.

2. Condensed tannin analyses

2.1. Chemicals

Hydrochloric (37%, analytical reagent grade), acetone (analytical reagent grade), butan-1-ol (standard laboratory reagent grade), acetonitrile (HPLC grade), formic acid (HPLC grade), methanol (HPLC grade) were obtained from Fisher Scientific (Loughborough, UK); benzyl mercaptan (BM) from Sigma-Aldrich (Poole, UK); ultrapure water (MQ H_2O) from a Milli-Q Plus system (Millipore, Watford, UK).

2.2. Tannin analysis by acetone-HCl-butanol assay

The method was performed according to Grabber et al. (2013). All samples and a positive control with each batch of samples (a standard *Onobrychis viciifolia* sample) were run in triplicate. Briefly, 10 mg of sample [*e.g.* either feed (sainfoin pellets, control pellets or hazelnut skin) or freeze-dried digesta or faeces samples] were weighed into screw capped tubes, 10 mL volume (Fischer Scientific, Loughborough, UK), and added 10 mL of reagent (150 mg ammonium ferric sulphate dodecahydrate, 3.3 mL water, 5.0 ml 12M HCl, 42 mL butan-1-ol, 50 mL acetone), a magnetic stirrer bar was added as well. The tubes were left at room temperature for 1 hour and afterward heated at 70°C for 2.5 hours in the dark (dry block heater SBH130D, Bibby Scientific Limited, Stone, UK). Then, the tubes were cooled down to room temperature, centrifuged (3000 g; Centrifuge 5702, Eppendorf®, 129 Hamburg, Germany) and transferred (3 mL of supernatant of each sample) to a quartz spectrophotometer cuvette. The colorimetric reaction was run in a spectrophotometer (Jasco V-530, Jasco UK, Dunmow, UK) set to record spectra from 450 to 650 nm. The acetone-HCl-butanol reagent was used as a blank, all the samples were run in triplicate. The absorbance at the peak apex was measured manually from these spectra. The CT (or proanthocyanidin) content was expressed as g/100 g of dry weight (DW).

2.3. Tannin analysis by thiolytic degradation and HPLC

The thiolysis reactions were carried out as described previously (Gea et al., 2011) with some minor changes. The features measured were CT contents (% CT) and features [(size in terms of mean degree of polymerization, mDP; percentage of prodelphinidins (PD) and procyanidins (PC) within CTs; and percentage of *trans*- vs *cis*-flavanols, % *trans* and % *cis*]. Freeze-dried samples (200 mg) were weighed into 10 mL glass tubes, methanol (2 mL) was added, followed by acidified methanol (1 mL of 3.3% HCl/ in MeOH), benzyl mercaptan (BM, 100 µl) and a magnetic stirrer. The tube was capped and heated in a water bath (ETS-D5, RCT basic, IKA®, Staufen, Germany) at 40 ˚C for 1 h under vigorous stirring.

Formic acid 1% in water (9 mL) was added to stop the reaction. Taxifolin 0.05 mg/mL was used as external standard. The samples were analysed within 24 hours, using an Agilent 1100 series HPLC system consisting of a G1315A degasser, G13112A binary pump, a G1313A ALS autoinjector, a G1316A column oven, a GI315A Diode Array Detector and a G1946B mass spectrometer (Agilent Technologies, Waldbronn, Germany). The column was an ACE super 18 column (5 µm; 150 x 3.0 mm; Hichrom Ltd., Theale, UK) fitted with ACE guard column. Data was acquired using ChemStation software (version A 10.01 Rev. B.01.03). The injection volume was set to 5 µL and the flow rate to 0.4 mL/min. The sample was eluted using a gradient of 1% formic acid in MilliQ H_2O containing 100 mg/L of ammonium chloride (solvent A) and 1% formic acid in HPLC-grade acetonitrile (solvent B) as follows: 0 – 7 min, isocratic 2.5 % B; 7-15 min, 2.5-5% B; 15-22 min, 5-10% B; 22-40 min, 10-40% B, which was followed by 40-45 min, 40-100% B; and return to 2.5% B at 49 min. Total cycle time was 60 minutes with the column oven et to 60 °C; chromatograms were recorded at 280 nm. Mass spectra were acquired in negative ionization mode with a capillary voltage of 3000V. Terminal and extension units were identified by their retention times, DAD spectra and the m/z ratio of the compounds which formed chloride adducts under these conditions. The peak areas at 280 nm were used for quantification as described Gea et al. (2011).

3. Statistical Analyses

Results are presented as mean values ± SD. Non-parametric analysis (Kruskal-Wallis and Kolmogorov-Smirnov test) were applied to either CT values per sort of sample (*i.e.* digesta or faces) measured with each analytical method (acetone-HCl-butanol, thiolysis), and flavan-3-ols composition. The comparisons concerned 1) the different diet treatments, 2) for each group, the difference between segments of the digestive tract. All statistical analyses were performed using Systat® 9 software (SPSS Ltd).

Results

1. Condensed tannin quantification in digesta samples and faeces

The values of CT measured by acetone-HCl-butanol, are presented in the Table 1. The CT content values for sainfoin pellets (SG) and hazelnut skin were respectively, 6.5 ± 0.3 (HG) 5.1 ± 0.1 [expressed as g of proanthocyanidins per 100 g of dry weight (g PA/100 g DW)]. No differences were observed for these values of CT ($P > 0.05$). It was not possible (as expected) to measure any CT in the control pellets which was supposed to be tannin-free.

Compared to the CT content value per feed, decrease in CT values were observed per digesta samples in either SG or HG lambs group. Thus, in SG samples the amount of CT detected in digesta samples or faeces were lower than in the feed (*i.e.* from 1.0 to 2.1 g PA/100 g). Therefore, compared to feed values, reduction in CT detected values were 84.6%, 67.7%, 72.4% and 69.2% for rumen, abomasum, small intestine and faeces, respectively in SG. Meanwhile, for HG samples, CT values ranged from 1.1 to 2.0 g PA/100 g, and the losses were: 78.5%, 66.7%, 76.5% and 60.8% for rumen, abomasum, small intestine and faeces, respectively. Overall, the CT measured demonstrated a similar pattern in SG and HG groups, with higher CT values observed in the abomasal digesta samples or faeces, whereas lower CT values were observed in either the rumen or small intestine digesta samples. No differences were observed in CT content values between HG and SG groups neither per sort of digesta sample nor in faeces ($P > 0.05$).

The CT content measured by thiolysis (Table 1) in the feed were, for sainfoin pellets 1.71 ± 1.01, and for hazelnut skin 6.3 ± 1.01 g PA/100 g DW, with statistical differences between them ($P < 0.01$). In the different organs along the gut in the SG group, the highest CT mean value was observed in the abomasal digesta sample (0.7±0.1 g PA/100 g DW), instead in the HG, both abomasal digesta sample and faeces showed the highest CT values (approx. 0.7±0.1 g PA/100 g DW in both cases). Hence, there was a large amount of non-detectable CT with thiolysis in digesta or faeces samples compared with the feed. Thus, the non-detected CT (in regard of the CT content of sainfoin pellets) in the SG were 85.3%, 58.8%, 76.5% and 76.5%, whereas for HG, the values were 92.1%, 88.9%, 93.7% and 88.9% in rumen, abomasum, small intestine or faeces, respectively. No differences were recorded for the CT values measured by thiolysis ($P > 0.05$) between groups nor sort of sample. In a similar way to the acetone-HCl-butanol assay, with the thiolysis, no CT content was detected in the samples from the control animals.

2. CT structure features in digesta samples and faeces

Overall, the CT structural features for the SG samples are presented in Table 2. In the digesta samples, it was not possible to evaluate the mDP values. Similar to the sainfoin pellets, overall high PD and high *cis* percentages were observed in all the samples with variations depending on the location along the gut.

Overall, in the HG group, the structure of CT seems preserved except for some changes in the faeces with lower PD% (Table 3). However, as in the hazelnut skin feed, high percentage of PC was observed in all the digestive samples. In regard to the mDP, no differences were observed in the various sort of samples. The *cis* % was reduced from 58% to values close or under 50% in the digesta and faeces.

3. CT flavan-3-ol monomer composition in digesta samples and faeces

CT composition with flavan-3-ol monomers from SG are presented in Table 4. Monomers of PD [*i.e.* gallocatechin (GC) and epigallocatechin (EGC)] and PC [*i.e.* catechin (C) and epicatechin (EC)] were observed. However, the absence of galloylated groups is noticeable. In terminal units, the flavanols GC or EGC were not observed in the samples although both were present in the sainfoin pellets. Instead, for the extension units, the monomer composition was close to the sainfoin pellets, except that catechin was not observed in faecal samples. The main flavan-3-ol in terminal units were C and EC, whereas in extension units, it was EGC-BM (62.7-75.7%). No differences were observed between samples in flavanols in terminal units ($P > 0.05$), but variations were recorded in extension units: GC-BM, ECG-BM and EC-BM.

In the HG samples (Table 5), the monomer composition showed slight variations, mainly in the extension units. Overall, in terminal units the component was C (6.5-7.6%), as in the feed (7.5%). Moreover, in the feed with hazelnut skins, some galloylated monomers [*i.e.* epicatechin gallate (ECG) and epigallocatechin gallate (EGCG)] were observed in the extension units (*i.e.* representing ≈ 5% of CT composition). Compared to the feed, the main changes observed were i) the disappearance of the galloylated monomers and ii) variations in the proportion of PD (EGC-BM + GC-BM) and PC (C-BM + EC-BM) particularly between faeces and the other samples.

Discussion

The current study was carried out in order to determine the possible changes in quantity and quality (structure) of CT during their transit through the digestive tract, with the aim of better understanding their bioavailability and related AH effects. Thus, lambs were fed with CT-containing resources with discriminant chemical structure, in regard of monomer composition [*i.e.* either with sainfoin pellets (SG), with a high PD/PC ratio composition or hazelnut skin (HG), with a low PD/PC ratio]. Such feature enabled us to assess if any qualitative variation occurs in regard of chemical structure and if the CT quantity varies between digestive compartments and therefore if their bioavalaibility and/or bioactivity may be affected. The measurements of CT content and chemical characterization were performed in digesta samples [*i.e.* from rumen, abomasum, small intestine (ileum)] and faeces and compared with the quantity and structural features of CT of the respective feed.

So far, few studies have been conducted in order to evaluate the bioavailability of CT in small ruminants. They were performed using a standard HCl-butanol assay (Perez-Maldonado and Norton, 1996; Terrill et al., 1994), and they were not related to parasite infections. In this line, only one recent study has reported CT's content and structure in digesta samples from GINs infected cattle fed with sainfoin and measuring CT with thiolysis (Desrues et al., 2015). To the best of our knowledge, the current study is the first report of CT content and CT structural characterization in digesta samples and faeces of lambs fed with two contrasting CT-resources (*i.e.* mainly PD or PC, sainfoin pellets or hazelnut skin, respectively).

The HCl-butanol assay has been defined as the most specific and commonly used spectrophotometric method for quantifying CT in plant tissues, foods and feeds (Mueller-Harvey, 2006). The protocol used here (with a reagent of acetone-HCl-butanol-iron) allows extraction and measurement of CT by up to 3.2-fold over the standard method run without acetone (Grabber et al., 2013).

Furthermore, in the current study, the thiolysis assay with benzyl mercaptan (BM), was applied to characterize the CTs. This assay provides, in addition of CT values, information on the structural features on the average composition of all tannins in terms of polymer size (mDP), PD/PC and *cis/trans* ratios (Gea et al., 2011). However, the thiolysis has some limitations, as discussed by Pérez-Jiménez and Torres (2011). The relatively mild acid treatment in the presence of nucleophiles compared to HCl-butanol assay may not release the same amount of CT from the sample matrix. In fact, it has been reported that some of the polymers resist degradation with thiols, as specifically described in plants which CTs originating from aged tissues (Matthews et al., 1997). Moreover, the extractability of tannins from plant tissues could be reduced by several factors (*e.g.* heating, exposure to UV, etc.), some of them being involved in the industrial processing of the sainfoin to produce the pellets as well as for the hazelnut, to obtain the skins (Silanikove et al., 2001).

Our CT values results measured by acetone-HCl-butanol in the digesta samples or faeces, showed a similar pattern as reported in an assay and digestion of ^{14}C-labelled CT in sheep, with considerable disappearance of CT in both the rumen and the small intestine (Terrill et al., 1994). Compared with the CT content observed in feed (*i.e.* sainfoin pellets or hazelnut skin), those obtained from either digesta samples or faeces were dramatically reduced in both SG and HG diets. Similarly, high percentage of disappearance in digesta or faeces samples were described previously in post-rumen losses of total CT in sheep (85 - 86%) (Perez-Maldonado and Norton, 1996; Terrill et al., 1994) and goats (83%)(Perez-Maldonado and Norton, 1996).

The CT values measured by thiolysis, were much lower than those obtained with the acetone-HCl-butanol method. As previously mentioned, this could be explained due the milder conditions used in this assay. Also Pérez-Jiménez and Torres (2011) and Ramsay et al. (2015) have mentioned that CTs had to be free to react to the benzyl-mercaptan. Therefore the complexation of CT with macromolecules could affect their detection. Nonetheless, in our results similar patterns were observed in regard of CT concentration per compartment with either acetone-HCl-butanol or thiolysis assays.

In the current results, although the CT values measured by thiolysis were similar between groups, much higher losses in CT values were observed in the HG than in the SG. This observation is related with the fact that the CT value of hazelnut skin (6.3 g PA/100 g DW) was higher than such measured in sainfoin pellets (1.7 g PA/100 g DW).

The same pattern of CT values and large differences (losses) between compartments were observed for both experimental groups, regardless of the assay applied to measure the CT. The highest values were measured in the abomasum in both groups and in faeces with hazel nut. However, in cattle a different pattern of CT was found along the gut. This result suggest that some differences in the CT extractability from digesta samples might occur between species of ruminant (Desrues et al., 2015).

On the other hand, in regard to CT quality or structure observed, the thiolysis performed to the feed samples, showed for SG, a CT structure with mainly PD monomer units, whereas for the HG composition, the PC units were the main flavanols, as reported previously (Del Rio et al., 2011; Lorenz et al., 2014; Wang et al; 2015). The CT structure features (*i.e.* mDP, PD/PC and *cis/trans* ratio) in the samples from both groups appeared overall, preserved with minor variations in feces, especially for the PD/PC ratio. The method applied allowed extraction and identification of terminal and extension units, except for the samples in the SG, in which the terminal units detected were only traces.

In the analysis of CT flavan-3-ol units, some compositional changes were observed in both experimental groups. These, suggest that the CT underwent some changes all along their transit through the digestive tract, irrespectively of the initial quality (*i.e.* high or low PD/PC ratio). For instance, in the SG samples of digesta and faeces, no GC nor EGC in the terminal units were detected anymore. In the HG, only traces of galloylated flavanols were detected in a few samples, whereas present in the feed. These may be a consequence of the high protein-binding capacity of galloylated groups (Hagerman, 2012; Le Bourvellec et al., 2005; Touriño et al., 2008), but also to their ease of hydrolysis.

Despite the fact that sainfoin pellets and hazelnut skin had relatively similar CT contents (*i.e.* measured by the acetone-HCl-butanol assay) and mDP values (*i.e.* sainfoin pellets 11.5, hazelnut skin 13.3), they appear to have different fates in the digestive tract. As the free CT are relatively large polymers to be absorbed, their apparent disappearance in digesta and faeces samples, could be related to i) either degradation and subsequent flavanol monomer units absorption (Gladine et al., 2007; López-Andrés et al., 2013) and/or ii) conformational changes in the CT structure (Choy et al., 2014; Perez-Maldonado and Norton, 1996; Terrill et al., 1994) and iii) complexation and binding to proteins and other macromolecules (Perez-Maldonado and Norton, 1996; Petra and Saxena, 2011). In addition to either sainfoin pellets (SG) or hazelnut skin (HG), lambs were fed with grass hay which could provide an additional suitable matrix to bind CT in ways that no longer allow their extraction (Le Bourvellec et al., 2005; Simon et al., 2003)

The bioavailability of phenolic compounds in ruminants has been scarcely investigated and most of the studies concerned monogastrics. For instance, in humans, it has been reported that CT are catabolized into phenolic acids of low molecular weight by colonic microflora (Déprez et al., 2000). In addition, it has been suggested that CTs are hydrolyzed by gastric fluids to lower molecular compounds (*i.e.* catechin and epicatechin) (Spencer et al., 2000). However, it was not confirmed further when Rios et al. (2003) observed that procyanidins are stable during gastric transit. On the other hand, in rats dosed by gavage with CT from carob (*Ceratonia siliqua*), only 25% of ^{14}C in the form of soluble tannins were found within the gut contents, whereas evidence of CT degradation/absortion and/or their binding to other components of the gut contents were reported (Abia and Fry, 2001). However, it was also reported that CT are not decomposed into bioavailable monomers in the rats' monogastric digestive system and it was concluded that only the monomeric components of the feed (*i.e.* catechin and epicatechin) were absorbed in monogastrics (Nakamura et al., 2003).

Notwithstanding, it seems that phenolic compounds are metabolized by different pathway in ruminant vs. monogastric. Bhat et al. (1998), mentioned that the ruminal microflora was able to degrade quebracho tannins into smaller phenolic compounds. Gladine et al. (2007) found monomeric phenolic compounds in plasma when the sheep had received polyphenol-rich plant extracts (*i.e.* grape or rosemary) by ruminal infusion. Finally, in both monogatrics and ruminants, some evidence suggest that the colonic microbiota might play an important role as has been mentioned in human (Ward et al., 2004), rats (Gonthier et al., 2003) and sheep and goats (Pérez-Maldonado and Norton, 1996).

However, another degradation pathway may be involved, since it is believed that procyanidins are directly degraded to phenolic acids, without previous depolymerization into C and/or EC units (Appeldoorn et al., 2009). Also, polyphenols may undergo spontaneous oxidation as mentioned by Hagerman (2012). Therefore, CT are not inert within the gut but undergo various modifications (Abia and Fry, 2001).

In our data, the highest CT values were obtained in abomasal digesta and the lowest from the rumen, irrespective of feed group. As explained by (Mueller-Harvey, 2006), the CT bind proteins in conditions close to neutral pH. Thus CT-protein complexes may be formed in rumen, then, under the acid conditions in the abomasum ($pH < 3.5$) or alkaline in the intestine ($pH > 7$) be released (Terrill et al., 1994). Indeed, our results agree with the fact that CT bind protein and that complexation may partially explain the variation in detectable CT in the digesta samples according to the organ where they were taken. The increased CT values in faeces may be explained as the synergistic action exerted by the pH (> 7) and bile salts in disrupting the CT-protein complexes through the small intestine (Perez-Maldonado and Norton, 1996). It has been reported that CT may interact with proteins but also with carbohydrates, lipids and intestinal mucosa (Patra and Saxena, 2011; van Leeuwen et al., 1995). Also it is worth to consider that all the non-absorbed components in the digesta are concentrated in faeces after the digestive processes (Gedir et al., 2005).

The CT complexation with proteins [*i.e.* exogenous, from the feed or endogenous (*e.g.* enzymes, intestinal mucosa)] seems to be related with their structure. For instance mDP (size) and monomer units are pointed as key factors (Stojadinovic et al., 2013) but the protein three-dimensional structure seems to play a more important role (Lorenz et al., 2014; Simon et al., 2003). Simon et al. (2003) reported that one molecule of peptide is able to bind three CT molecules. This finding may explain the nature of intricate aggregates that avoid CT to be extracted from complex matrix samples as digesta or faeces.

It has been suggested that this property accounts for the AH effects observed of CT. For instance, in adult *H. contortus* exposed *in vivo* and *in vitro* to CT-extracts, aggregates on the nematode surface have been observed (Martínez-Ortíz-de-Montellano et al., 2013). The nematode cuticle, but also secretion/excretion products (*i.e.* enzymes, etc.) are protein rich (Page, 2001). It has been proposed that a direct effect of CT complexation with nematode proteins, as reported by Martínez-Ortíz-de-Montellano et al. (2013) might affect the parasite's physiology (feeding, mobility, reproduction, copula, excretion)(Hoste et al., 2012, 2015). Additionally, in ensheathed and exsheathed infective larvae of *H. contortus* and *T. colubriformis* exposed to CTs, lesions have also been observed (Brunet et al., 2011) on the larvae surface but also in the intestine. Therefore, in regard of CT, it seems that the higher protein binding capacity they have, the better AH effect they might exert. Actually, the higher protein-binding flavan-3-ols EGCg and GCg showed *in vitro* the stronger AH effect for *H. contortus* and especially for *T. colubriformis* (Brunet and Hoste, 2006). Both monomers are prodelphinidins (PD), and the galloylated flavan-3-ols are pointed as more efficient to precipitate proteins (Hagerman, 2012). The mDP of CTs also influence protein precipitation (Stojadinovic et al., 2013). Hagerman (2012), mentioned that CT precipitability increased with increased mDP. This structural feature has recently been reported as a key variable for the *in vitro* AH effect against parasites of cattle (Novobilský et al. 2013), pigs (Williams et al., 2014) and small ruminants (Quijada et al., 2015).

Conclusions

In the current study, the CT content values observed in either digesta or faeces were much lower than those present in the feed [*i.e.* sainfoin pellets (SG) or hazelnut skin (HG)], and showed similar patterns by digestive compartment, irrespective of the CT quality [*i.e.* high (sainfoin pellets) or low (hazelnut skin) PD/PC ratio)] of the two CT-containing feed offered to the lambs. The CT amount in faeces was higher than those observed in small intestine or rumen but still much lower that the content in feed. This finding led to hypothesize that the disappearance of CT through the gastrointestinal tract could be related to conformational changes in regard of their complexation with various sort of macromolecules present in digesta. Therefore, such conformational changes may make CT no longer detectable by either colorimetric or thiolysis methods. However, due to the large CT disappearance, degradation and/or absorption may not be discarded.

On the basis of the present results, it seems that the CTs in digesta or faeces from sheep underwent high variations in regard of concentration (quantity) during their passage through the gastrointestinal tract. In contrast, the structure features (quality) seemed overall preserved in both groups, whereas the flavan-3-ol composition showed only slight variations in both groups. Further studies are needed to reveal the actual impact of these CT reactivies in the AH activity.

Abbreviations

PSM, plant secondary metabolite; HT, hydrolysable tannin; CT, condensed tannin; PA, proanthocyanidin; AH, anthelmintic; GIN, gastrointestinal nematode; GI, gastrointestinal; PD, prodelphinidin; PC, procyanidin; mDP, mean degree of polymerization; BM, benzyl mercaptan; NEP, nonextractable polyphenols; NEPA, nonextractable proanthocyanidin; SG, sainfoin pellets lambs group; HS, hazelnut skin lambs group; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGC, epigallocatechin; EGCG, epigallocatechin gallate; GC, gallocatechin; GCG, gallocatechin gallate.

Acknowledgements

The financial support of the European Commission through the PITN-GA-2011-289377, "LegumePlus" project is sincerely acknowledged. Assistance from Mrs. Fabienne Picard and Mr. Vincent Niderkorn (INRA, UMR 1213 Herbivores, Saint-Genès-Campanelle, France) for freeze-drying of digesta samples is deeply appreciated.

References

Abia, R., Fry, S.C., 2001. Degradation and metabolism of ^{14}C-labelled proanthocyanidins from carob (*Ceratonia siliqua*) pods in the gastrointestinal tract of the rat. *J. Sci. Food Agric.*, 81, 1156–1165.

Appeldoorn, M.M., Vincken, J.P., Aura, A. M., Hollman, P.C., Gruppen, H., 2009. Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl) acetic acid and 5-(3,4-dihydroxyphenyl)-gamma-valerolactone as the major metabolites. *J. Agric. Food. Chem.*, 57, 1084–1092.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.*, 99, 205–219.

Bhat T.K., Singh B., Sharma O.P. 1998. Microbial degradation of tannins – a current perspective. *Biodegradation*, 9, 343–357.

Bhatta, R., Saravanan, M., Baruah, L., Prasad, C.S., 2015. Effects of graded levels of tannin-containing tropical tree leaves on *in vitro* rumen fermentation, total protozoa and methane production. *J. Appl. Microbiol.*, 118, 557–564.

Brunet, S., Aufrère, J., El Babili, F., Fouraste, I., 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., Hoste, H., 2011. Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (*Onobrychis viciifolia*) extract. *Parasitol. Int.*, 60, 419–424.

Brunet, S., Hoste, H., 2006. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. *J. Agric. Food Chem.*,54, 7481–7.

Buccioni, A., Pauselli, M., Viti, C., Minieri, S., Pallara, G., Roscini, V., Rapaccini, S., Marinucci, M.T., Lupi, P., Conte, G., Mele, M., 2015. Milk fatty acid composition, rumen microbial population, and animal performances in response to diets rich in linoleic acid supplemented with chestnut or quebracho tannins in dairy ewes. *J. Dairy Sci.*, 98, 1145–56.

Burke, J.M., Whitley, N.C., Pollard, D. a, Miller, J.E., Terrill, T.H., Moulton, K.E., Mosjidis, J. a, 2011. Dose titration of sericea lespedeza leaf meal on *Haemonchus contortus* infection in lambs and kids. *Vet. Parasitol.*, 181, 345–9.

Choy, Y.Y., Quifer-Rada, P., Holstege, D.M., Frese, S. A., Calvert, C.C., Mills, D. A., Lamuela-Raventos, R.M., Waterhouse, A.L., 2014. Phenolic metabolites and substantial microbiome changes in pig feces by ingesting grape seed proanthocyanidins. *Food Funct.*, 5, 2298–2308.

Del Rio, D., Calani, L., Dall'Asta, M., Brighenti, F., 2011. Polyphenolic composition of hazelnut skin. *J. Agric. Food Chem.*, 59, 9935–9941.

Desrues, O., Peña-Espinoza, M., Hansen, T.V., Mueller-Harvey, I., Enemark, H.L., Thamsborg, S.M., 2015. Anthelmintic effects of sainfoin against different cattle nematodes may be linked to concentration of condensed tannins in different gut compartments. 25th WAAVP conference. Liverpool, August 2015.

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey, I., 2011. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. *J. Agric. Food Chem.*, 59, 495–503.

Gedir, J. V., Sporns, P., Hudson, R.J., 2005. Extraction of condensed tannins from cervid feed and feces and quantification using a radial diffusion assay. *J. Chem. Ecol.*, 31, 2761–2773.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D., 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *Br. J. Nutr.*, 98, 691–701.

Grabber, J.H., Zeller, W.E., Mueller-Harvey, I., 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in lotus species by the butanol-HCl-iron assay. *J. Agric. Food Chem.*, 61, 2669–2678.

Gonthier M. P., Donovan J. L., Texier O., Felgines C., Rémésy, C., Scalbert A. 2003. Metabolism of dietary procyanidins in rats. *Free Radical Biol. Med.*, 35, 837–844.

Hagerman, A. 2012. Fifty years of polyphenol-protein complexes. In: Recent advances in polyphenol research. 1st Edition. Cheynier, V.; Sami-Machado, P.; Quideau, S. Eds. Publisher: Wiley-Blackwell & sons, Oxford, UK. Vol. No. 3. 364 pp.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Vet. Parasitol.*, 186, 18–27.

Hoste, H., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., Terrill, T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. *Vet. Parasitol*., 212, 5–17.

Hoste, H., Torres-Acosta, J.F.J., Quijada, J., Chan Perez, I., Dakheel, M.M., Kommuru, D.S, Mueller-Harvey, I., Terrill, T.H. Interactions between nutrition and infections with *H. contortus* (and related gastro intestinal nematodes) in small ruminants. *Adv. Parasit.* (in press).

Le Bourvellec, C., Bouchet, B., Renard, C.M.G.C., 2005. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochim. Biophys. Acta - Gen. Subj.*, 1725, 10–18.

Liu, H., Vaddella, V., Zhou, D., 2011. Effects of chestnut tannins and coconut oil on growth performance, methane emission, ruminal fermentation, and microbial populations in sheep. *J. Dairy Sci.*, 94, 6069–77.

López-Andrés, P., Luciano, G., Vasta, V., Gibson, T.M., Biondi, L., Priolo, A., Mueller-Harvey, I., 2013. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. *Br. J. Nutr.*, 110, 632–9.

Lorenz, M.M., Alkhafadji, L., Stringano, E., Nilsson, S., Mueller-Harvey, I., Udén, P., 2014. Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. *J. Sci. Food Agric*., 94, 963–968.

Luciano, G., Vasta, V., Monahan, F.J., López-Andrés, P., Biondi, L., Lanza, M., Priolo, A., 2011. Antioxidant status, colour stability and myoglobin resistance to oxidation of *longissimus dorsi* muscle from lambs fed a tannin-containing diet. *Food Chem.*, 124, 1036–1042.

Martínez-Ortíz-de-Montellano, C., Arroyo-López, C., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Hoste, H., 2013. Scanning electron microscopy of *Haemonchus contortus* exposed to tannin-rich plants under *in vivo* and *in vitro* conditions. *Exp. Parasitol.*, 133, 281–6.

Matthews, S., Mila, I., Scalbert, A., Pollet, B., Lapierre, C., duPenhoat, C., Rolando, C., Donnelly, D.M.X., 1997. Method for estimation of proanthocyanidins based on their acid depolymerization in the presence of nucleophiles. *J. Agric. Food Chem.*, 45, 1195–1201.

Moreno-Gonzalo, J., Manolaraki, F., Frutos, P., Hervás, G., Celaya, R., Osoro, K., Ortega-Mora, L.M., Hoste, H., Ferre, I., 2013a. *In vitro* effect of heather extracts on *Trichostrongylus colubriformis* eggs, larvae and adults. *Vet. Parasitol.*, 197, 586–594.

Moreno-Gonzalo, J., Manolaraki, F., Frutos, P., Hervás, G., Celaya, R., Osoro, K., Ortega-Mora, L.M., Hoste, H., Ferre, I., 2013b. I*n vitro* effect of heather (Ericaceae) extracts on different development stages of *Teladorsagia circumcincta* and *Haemonchus contortus*. *Vet. Parasitol.*, 197, 235–243.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric*., 86, 2010–2037.

Nakamura Y., Tonogai Y. 2003. Metabolism of grape seed polyphenols in the rat. *J. Agric. Food Chem.*, 51, 7215–7225.

Naumann, H.D., Armstrong, S. a., Lambert, B.D., Muir, J.P., Tedeschi, L.O., Kothmann, M.M., 2014. Effect of molecular weight and concentration of legume condensed tannins on *in vitro* larval migration inhibition of *Haemonchus contortus*. *Vet. Parasitol.*, 199, 93–98.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. *Vet. Parasitol.*, 182, 213–20.

Novobilský, A., Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Enemark, H.L., Mueller-Harvey, I., Thamsborg, S.M., 2013. *In vitro* effects of extracts and purified tannins of sainfoin (*Onobrychis viciifolia*) against two cattle nematodes. *Vet. Parasitol.*, 196, 532–7.

Page, A.P., 2001. The nematode cuticle: synthesis, modification and mutants. In: Parasitic nematodes: molecular biology, biochemistry and immunology, Kennedy and Harnett (Eds.), London, pp. 167-193.

Paolini, V., Bergeaud, J.P., Grisez, C., Prevot, F., Dorchies, P., Hoste, H., 2003a. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Vet. Parasitol.*, 113, 253–261.

Paolini, V., Frayssines, A., Farge, F., Dorchies, P., 2003b. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Vet. Res.*, 34, 331–339.

Patra, A.K., Saxena, J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric.*, 91, 24–37.

Pérez-Jiménez, J., Torres, J.L., 2011. Analysis of non-extractable polyphenols in foods: the current sate of the art. *J. Agric. Food Chem.*, 59, 12713–12724.

Perez-Maldonado, R. a, Norton, B.W., 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. *Br. J. Nutr.*, 76, 515–533.

Quijada, J., Fryganas, C., Ropiak, H.M., Ramsay, A., Mueller-Harvey, I., Hoste, H., 2015. Anthelmintic activities against *Haemonchus contortus* or *Trichostrongylus colubriformis* from small ruminants are influenced by structural features of condensed tannins. *J. Agric. Food Chem.*, 63, 6346–6354.

Ramsay, A., Drake, C., Grosse Brinkhaus, A., Girard, M., Copani, G., Dohme-Meier, F., Bee, G., Niderkorn, V., Mueller-Harvey, I., 2015. Sodium hydroxide enhances extractability and analysis of proanthocyanidins in ensiled sainfoin (*Onobrychis viciifolia*). *J. Agric. Food Chem.*, 63, 9471–9479.

Rios L. Y., Gonthier M. P., Rémésy C., Mila I., Lapierre C., Lazarus S. A., Williamson G., Scalbert A. 2003. Chocolate intake increases urinary excretion of polyphenol-derived phenolic acids in healthy human subjects. *Am. J. Clin. Nutr*., 77, 912–918.

Salminen, J.P., Karonen, M., Sinkkonen, J., 2011. Chemical ecology of tannins: Recent developments in tannin chemistry reveal new structures and structure-activity patterns. *Chem. - A Eur. J.*, 17, 2806–2816.

Silanikove, N., Perevolotsky, A., Provenza, F.D., 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Anim. Feed Sci. Technol.*, 91, 69–81.

Simon, C., Barathieu, K., Laguerre, M., Schmitter, J.M., Fouquet, E., Pianet, I., Dufourc, E.J., 2003. Three-dimensional structure and dynamics of wine tannin-saliva protein complexes. A multitechnique approach. *Biochemistry*, 42, 10385–10395.

Spencer J.P.E., Chaudry F., Pannala A.S., Srai S.K., Debnam A., Rice-Evans, C. 2000. Decomposition of cocoa procyanidins in the gastric milieu. *Biochem. Biophys. Res. Com*., 272, 236–241.

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., Cirkovic Velickovic, T., 2013. Binding affinity between dietary polyphenols and B-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chem.*, 136, 1263–1271.

Terrill, T.H., Waghorn, G.C., Woolley, D.J., McNabb, W.C., Barry, T.N., 1994. Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep. *Br. J. Nutr*., 72, 467–477.

Touriño, S., Lizárraga, D., Carreras, A., Lorenzo, S., Ugartondo, V., Mitjans, M., Vinardell, M.P., Julía, L., Cascante, M., Torres, J.L., 2008. Highly galloylated tannin fractions from witch hazel (*Hamamelis virginiana*) bark: Electron transfer capacity, *in vitro* antioxidant activity, and effects on skin-related cells. *Chem. Res. Toxicol.*, 21, 696–704.

Wang, Y., Mc Allister, T.A., Acharya, S. Condensed tannins in sainfoin: composition, concentration, and effects on nutritive and feeding value of sainfoin forage. *Crop Sci.*, 55, 13-22;

Ward N. C., Croft K. D., Puddey I. B., Hodgson J. M. 2004. Supplementation with grape seed polyphenols results in increased urinary excretion of 3-hydroxyphenylpropionic acid, an important metabolite of proanthocyanidins in humans. *J. Agric. Food Chem.*, 52, 5545–5549.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One 9*, DOI: 10.1371/journal.pone.0097053

Zhong, R.Z., Li, H.Y., Sun, H.X., Zhou, D.W., 2014. Effects of supplementation with dietary green tea polyphenols on parasite resistance and acute phase protein response to *Haemonchus contortus* infection in lambs. *Vet. Parasitol.*, 205, 199–207.

Table 1. Condensed tannin content mean values (g PA/100 g DW) measured by the acetone-HCl-butanol assay and thiolysis method in digesta and faeces samples and feed in each experimental group (n= 5 lambs). [**(P < 0.01) indicates significant differences between sainfoin pellets and hazelnut skin feed. Different superscripts per line indicate, for the same diet, significant differences depending on the digestive organs or faeces]

	Feed	Rumen	Abomasum	Small Intestine	Faeces
acetone-HCl-butanol assay					
Sainfoin pellets group	6.5±0.3^{a}	1.0±0.1^{b}	2.1±0.3^{c}	1.8±0.3^{b}	2.0±0.4^{b}
Hazelnut skin group	5.1±0.1^{a}	1.1±0.1^{b}	1.7±0.2^{bc}	1.2±0.1^{bc}	2.0±0.3^{c}
Thiolysis method					
Sainfoin pellets group	1.71±0.07^{a**}	0.25±0.02^{b}	0.70±0.1^{c}	0.36±0.08^{bc}	0.39±0.0^{b}
Hazelnut skin group	6.27±0.02^{a**}	0.51±0.08^{b}	0.74±0.0^{b}	0.40±0.08^{b}	0.72±0.1^{b}

Table 2. Chemical characterization of digesta or faeces samples from SG lambs (n= 5) and sainfoin pellets. mDP (mean degree of polymerization); PC (procyanidin); PD (prodelphinidin); *cis* or *trans* (stereoisomerism of flavan-3-ol monomers). No significant differences were observed for the different measurements in the different organs.

	mDP	PC %	PD %	*cis %*	*trans %*
Sainfoin pellets	11.5±0.3	25.2±0.5	74.8±0.5	85.3±0.1	14.7±0.1
Rumen	-	20.5±0.9	79.5±0.9	87.9±0.7	12.1±0.7
Abomasum	-	15.9±0.5	84.1±0.5	91.3±0.5	8.7±0.5
Small intestine	-	21.3±1.1	78.7±1.1	87.5±0.3	12.5±0.3
Faeces	-	27.6±1.6	72.4±1.6	88.9±1.3	11.1±1.3

Table 3. Chemical characterization of digesta or faeces samples from HG lambs (n= 5) and hazelnut skin. mDP (mean degree of polymerization); PC (procyanidin); PD (prodelphinidin); *cis* or *trans* (stereoisomerism of flavan-3-ol monomers). No significant differences were observed for the different measurements in the different organs.

	mDP	PC %	PD %	*cis %*	*trans %*
Hazelnut skin	13.3±0.1	72.1±0.2	27.9±0.2	58.4±0.2	41.6±0.2
Rumen	14.8±0.7	65.6±1.5	34.3±1.5	46.3±1.2	53.7±1.2
Abomasum	13.9±0.3	66.6±0.7	33.4±0.7	51.3±0.6	48.7±0.6
Small intestine	13.8±1.2	66.7±1.7	33.4±1.7	46.9±2.3	53.1±3.3
Faeces	13.2±0.3	81.2±2.4	18.9±2.4	48.4±0.6	51.7±0.6

Table 4. Flavan-3-ol composition (%) of CT from SG lambs samples and sainfoin pellets. (P > 0.05). (GC, gallocatechin; EGC, epigallocatechin; C, catechin; EC, epicatechin; BM, benzyl mercaptan, is a nucleophile which bind to extension units during the thiolysis)

	terminal units				extension units			
	GC	EGC	C	EC	GC-BM	EGC-BM	C-BM	EC-BM
Sainfoin pellets	2.4±0.1	1.8±0.1	1.9±0.1	2.7±0.1	9.5±0.3	61.2±0.5	0.9±0.1	19.7±0.3
Rumen	0.0	0.0	0.0	0.0	10.3±0.5	69.2±1.5	1.7±0.1	18.8±0.9
Abomasum	0.0	0.0	1.3±0.0	1.2±0.0	8.3±0.3	75.7±0.7	0.6±0.0	15.4±0.5
Small intestine	0.0	0.0	0.0±0.0	1.9±0.0	11.9±0.3	66.7±1.5	0.5±0.0	19.8±1.9
Faeces	0.0	0.0	2.5±0.2	2.3±0.2	10.1±0.7	62.3±2.2	0.0	24.8±1.1

Table 5. Flavan-3-ol composition (%) of CT from HG lambs samples and hazelnut skin. (P > 0.05). (GC, gallocatechin; EGC, epigallocatechin; C, catechin; EC, epicatechin; ECG, epicatechin gallate; EGCG, epigallocatechin gallate; BM, benzyl mercaptan, is a nucleophile which bind to extension units during the thiolysis)

	terminal units				extension units					
	GC	EGC	C	EC	GC-BM	EGC-BM	C-BM	EC-BM	ECG-BM	EGCG-BM
Hazelnut Skin	0.0	0.0	7.5±0.1	0.0	12.1±0.1	15.1±0.1	21.2±0.1	39.4±0.3	0.87±0.1	3.99±0.1
Rumen	0.0	0.0	6.8±0.2	0.0	20.1±1.5	14.3±0.3	26.8±0.6	31.9±1.1	0.0	0.0
Abomasum	0.0	0.0	6.5±0.1	0.7±0.1	16.3±0.6	17.2±0.2	25.9±0.6	33.5±0.4	0.0	0.0
Small intestine	0.0	0.0	7.5±0.8	0.0	17.8±0.5	15.6±1.4	27.8±1.7	31.4±1.1	0.0	0.0
Faeces	0.0	0.0	7.6±0.2	0.0	10.1±1.7	8.8±0.9	34.0±1.0	39.5±1.3	0.0	0.0

DISCUSSION GENERALE

Several results from both *in vivo* and *in vitro* studies with condensed tannins (CT)-containing resources confirmed an AH activity against nematodes in small ruminants (Hoste et al., 2015; Hoste et al., in press). However, the variability between results led to hypothesize that there are factors related either to the nematodes species and/or to the polyphenolic compounds which influence the AH effect. For the nematodes, either intrinsic species susceptibility or different level of exposition to CTs have been proposed as main factors (Hoste et al., 2012). For CTs, both the quantity and/or the quality have been evoked as factors that influence the activity against parasitic nematodes. Indeed, some studies have suggested that different structural features of CTs are involved in their AH effects, namely:

i) the CT size (Barrau et al., 2005, Naumann et al., 2014; Novobilský et al., 2013; Williams et al., 2014)
ii) the type of flavan-3-ol subunits that give rise to either prodelphinidin (PD) or procyanidin tannins (PC) (Brunet and Hoste 2006; Molan et al., 2003, Molan et al., 2004, Williams et al., 2014)
iii) the stereochemistry of the C-ring in these subunits (*i.e. trans* vs. *cis* flavan-3-ols) (Molan et al., 2003, Naumann et al., 2014)
iv) since CTs and other polyphenolics (*i.e.* flavonoids) co-occur in plants, it is pertinent to investigate their combined interaction/potencies (Kozan et al., 2013; Yang et al., 2008).

A better understanding of the origin of variability in results observed with these plant compounds is required for a more rational use of these nutraceutical feeds under farm conditions (Hoste et al., 2015).

My thesis has had for general objective, to better understand the structure/activity relationship of CTs against parasite nematodes of small ruminants. The addressed hypotheses were that both quantitative and qualitative differences in CTs might influence the activity on different species of parasitic nematodes.

On the basis of these hypotheses, we performed *in vitro* and *in vivo* studies. For these studies, the abomasal species, *H. contortus,* was used as the main experimental model of parasitic nematode. However, as far as possible, the obtained results were compared with the small-intestinal worm, *T. colubriformis* in order to address the question of variability in results depending on the nematode species.

1. *In vitro* studies (Articles 1 et 2):

For these studies (Article 1 and Article 2), a wide range of purified CTs were evaluated to assess their AH effects against infective larvae of both *H. contortus* and *T. colubriformis*. The CTs extraction and fractionation were performed according to Stringano et al. (2011) with few modifications (Williams et al., 2014). Then, the purified CT fractions were subjected to thiolytic degradation as described by Gea et al. (2011) in order to analyze CT contents and quality (mDP, PD/PC and *cis*/*trans* flavan-3-ol ratios).

The method applied to measure the AH *in vitro* studies was the larval exsheathment inhibition assay (LEIA), which has been widely used to screen the AH activity of either plant extracts (Bahuaud et al., 2006; Jackson and Hoste, 2010), tannin fractions (Molan et al., 2004; Novobilský et al., 2011) or flavan-3-ol monomers (Brunet and Hoste 2006; Molan et al., 2003). The LEIA was chosen because it has been proven to be simple, reproducible and it allows calculations of EC50 values. Moreover, it is related to some *in vivo* processes (Brunet et al., 2007). The range of CT concentrations applied in the LEIA in both studies was from 37.5 to 600 µg fraction/mL. Such range was used in order to mimic physiological concentrations in ruminants according to values previously reported of extractable tannins ranging from 350 to 900 µg/mL in the sheep gut (Terrill et al., 1994).

For the first *in vitro* study (Article 1), 36 CTs fractions from 15 plants/resources were used (Article 1). They were selected because of their wide range of CT, mDP, PD/PC and *cis/trans* flavan-3-ol contents (ratios), in order to evaluate correlations between CTs structural features and the AH effects.

For the second *in vitro* study (Article 2), eigth CTs fractions (F1 and F2) from four plants with high (*i.e. Ribes nigrum*, *Ribes rubrum*) or low (*i.e. Salix caprae*, *Tilia* spp.) PD/PC ratios were obtained. The aim was to examine the occurrence of possible interactions with various flavonoids (*i.e.* arbutin, vanillic acid, taxifolin, naringenin, quercetin, luteolin) which are also present in the plants.

1.1. Structure/activity relationships of CTs against nematode parasites and synergistic effects with polyphenolics compounds:

- **CT content and AH activity:**

A dose-dependent effect was observed in the larval exsheathment inhibition in regard to the purified-CTs concentrations applied during both *in vitro* studies (Article 1 and

Article 2). However, overall, no correlations were observed between the CT contents and the AH activity against both parasite species. This finding agrees with several recent studies. Thus, Naumann et al. (2014) also found no relation between CT content and the AH activity against *H. contortus* L3 when comparing fractions from three legumes (*Lespedeza stuevei, L. cuneata* and *Arachis glabrata*). Novobilský et al. (2013) compared the effects of different CT fractions from *O. viciifolia* on cattle nematodes L1 (LFIA) of either the abomasum (*Ostertagia ostertagi*) or the small intestine (*Cooperia oncophora*). Again, they did not obtain consistent correlations between the CT contents and the *in vitro* AH activity.

This discrepancy in the dose-dependent relationship for AH activities observed between CT-containing extracts or fractions could be due to the AH properties of some other compounds of low molecular weight (phenolics and flavonoids) that can be present in extracts (Barrau et al., 2005; Brunet and Hoste 2006; Tibe et al., 2011) but not in purified CT fractions.

- **CT quality and AH activity:**

In our *in vitro* studies on the CTs structure/activity relationships, altogether lower EC50 (better AH effects) were assessed for *H. contortus* (Article 1 and Article 2) and *T. colubriformis* (Article 1) with the fractions with high PD/PC ratios. This also explains why the F2s, which possess higher PD/PC ratio compared to F1s, were overall more active. These results agree with recent findings on nematode parasite from cattle (Novobilsky et al., 2013) or pigs (Williams et al., 2014). It has been proposed that the biological activity is affected by the hydroxylation at the B-ring in flavan-3-ol monomers and polymers, where the presence of an additional hydroxyl group (OH) increases the interactions with proteins. This could explain the generally higher activity of PD rich tannins compared to the PC rich ones. Moreover, activity seems also increased when galloyl groups are present (Brunet and Hoste, 2006; Brunet et al., 2008; Li et al., 2010; Okuda and Ito, 2011).

Molan et al. (2003) also reported deleterious effects of flavan-3-ol monomers against *T. colubriformis* on different stages of the parasite life cycle. The highest AH effect occurred with the epigallocatechin gallate (EGCG) monomer. This observation was confirmed by further studies with green-tea fractions that were tested against *Teladorsagia circumcincta* and *T. colubriformis*. Again, higher EGCG content was linked with a higher AH effect (Molan et al., 2004). Similarly, when monomeric subunits of CT were tested by LEIA on *H. contortus* and *T. colubriformis* (Brunet and Hoste, 2006), a higher AH activity was observed with i) the monomeric subunits of PDs [*i.e.* gallocatechin (GC), epigallocatechin(EGC)] and ii) the galloyl derivatives of both prodelphinidins and procyanidins.

In regard to polymer size (mDP), negative correlations were observed between the EC50s and the mDP for *H. contortus* but not for *T. colubriformis* (Article 1). Similarly, Williams et al. (2014) reported a possible relation between the mDP values and the AH effect of CT against *Ascaris suum* L3s and Novobilský et al. (2013) against *Ostertagia ostertagi* and *Cooperia oncophora* L1s. Both studies, pointed out that the CT polymer size is an important factor in determining the AH activity, higher mDP being associated with increased efficacy (Williams et al., 2014).

Finally, no correlations were found between the *cis/trans* flavan-3-ols ratio and the EC50s (Article 1 and Article 2). The lack of relation between CT flavan-3-ols stereochemistry (configurational isomerism) and AH activity has also been reported before (Novobilský et al., 2013; Williams et al., 2014).

- **Synergistic AH effect of CT and flavonoid monomers:**

Our *in vitro* study described in Article 2 is the first report of synergistic effects between CTs and two common flavonoids from different groups [*i.e.* flavonols (quercetin) and flavones (luteolin)] based on the *in vitro* inhibition of exsheathment of *H. contortus* L3.

From preliminary screening test, the phenolic naringenin, quercetin and luteolin were chosen for further investigation in combination with different CTs. The additivity inhibitory effect was calculated using the definition of Bliss additivity (Bliss et al., 1939).

Synergy, is defined as the phenomena where two or more agents together produce an effect greater than would be predicted from their individual contributions. There are various mathematical models to assess synergy (Williamsom, 2001). Using the Bliss model (Williams et al., 2012), we observed synergistic effect between all the CTs and quercetin or luteolin in terms of AH activity during the exsheathment of *H. contortus* L3. However, it seems that the synergistic effects between CTs and quercetin (or luteolin) were more evident with tannins presenting low PD/PC ratio (from *Tilia* and *S. caprae)* than with tannins from *R. nigrum* and *R. rubrum* of high PD/PC ratio. This could be related to the fact that CT with lower PD/PC ratios offer more scope to enhancement as they tend to have lower AH activities than CT high PD/PC ratios (Brunet and Hoste, 2006; Novobilský et al., 2011).

The mechanism(s) of the observed synergistic effect are not identified but probably complex. The CT have been shown to deform nematode surfaces (Hoste et al. 2006, Martinez-Ortiz-de Montellano et al., 2013; Williams et al., 2014). Moreover, CT deleterious effects on infective larvae have also been observed (Brunet et al., 2011). These authors speculated whether the lesions in the cuticle of ensheathted L3 could have been due to some cellular toxicity by blocking the metabolic exchange with the environment. Quercetin is well known as an inhibitor of P-glycoproteins, which play an important role in the transport of xenobiotics (Lespine et al., 2012). One can question whether similar mechanisms are involved with polyphenols.

On the basis of these results, it seems that the AH effect of CT, especially those with low PD/PC ratio can benefit more from the addition of quercetin. Therefore, opportunities should be investigated for increasing the AH activity by mixing plant materials that contained CTs and quercetin or lutein flavonoids, or to select plants with enhanced CT and quercetin or luteolin contents.

- **CT *in vitro* AH effect per parasite species:**

To summarize, the results obtained from the *in vitro* studies (Article 1 and Article 2), showed a relation between the AH activity and the PD/PC ratio for both species. In regard of the EC50 values, a higher susceptibility was observed for *H. contortus* when compared with *T. colubriformis* (Article 1).

In regard of the lower EC50 measured for *H. contortus*, Molan et al. (2004) also pointed out that the abomasal nematode *T. circumcincta* was more susceptible than *T. colubriformis* to the AH effects of flavan-3-ol monomers and oligomeric CTs in the LMIA. The same conclusion was drawn from *in vitro* studies that examined extracts from different woody plants (*Rubus fructicosus, Quercus robur* and *Corylus avellana*) against *H. contortus, T. circumcincta* and *T. colubriformis* based on LMIA and LEIA tests (Paolini et al., 2004). However, other authors did not find such differences between abomasal and intestinal species in the response to quebracho or to *O. viciifolia* extracts (Athanasiadou et al. 2001; Brunet et al. 2007). Moreno-Gonzalo et al. (2013a, 2013b) even found a higher *in vitro* susceptibility (*i.e.* by calculating EC50, applying EHA and LEIA) of *T. colubriformis* compared to *H. contortus* and *T. circumcincta* when measuring the AH activity of extracts from different heather species (*Calluna vulgaris, Erica cinerea* and *E. umbellata*). It remains to be seen whether differences in assay conditions could account for some of these contradictory results. Nevertheless, the compared analysis of *in vitro* results presented in the Table 2 of the article submitted to *Advances in Parasitology* did not indicate clear intrinsic differences (higher susceptibility) between *H. contortus* and the other nematode species.

On the other hand, different CT structural features were also related with the AH effect against the 2 nematodes species. The main objective of the principals component analysis performed from the data generated in article 1 was to analyze the overall combined relationships between the different variables and the effects on exsheathment as assessed by the EC50 values. Results of these multivariate analyses for both GIN species tend to confirm the conclusions of the 2 by 2 Spearman correlations. For *Haemonchus*, the AH effects (EC50) were related with both the PD/PC ratio and the mDP, whereas for *Trichostrongylus* the PD/PC ratio appeared as the main factor.

These results suggest that some differences in mechanisms can be involved depending on the nematode species. For *H. contortus*, AH activity appeared stronger for CTs with higher PD contents and larger sizes (mDP values) since for the F2 fractions, lower EC50 values were associated with higher PD/PC ratio and larger tannins (higher mDP values). Novobilský et al. (2013) also suggested that mDP was a key factor in the LFIA against L3 of *O. ostertagi* and *C. oncophora* after testing *O. viciifolia* extracts and fractions. However, Naumann et al. (2014) did not find any clear evidence for a role of the CT size (*i.e.* mDP) on the inhibition of *H. contortus* motility, although only a narrow range of CT sizes was investigated. Conversely to the present data, Manolaraki (2011) found that lower mDP values were correlated with higher AH activity when extracts from forty *O. viciifolia* accessions were tested by LEIA against *H. contortus*. Similarly, Barrau et al. (2005) found that a fraction that contained CTs (< 2000 Da) plus flavonol glycosides had higher AH effects against *H. contortus* larvae than a fraction that contained only CTs (>2000 Da). Furthermore, as mentioned above (*vid. supra* "Synergistic AH effect of CT and flavonoid monomers"), the acetone/water extracts from CT-containing plants consist of CTs plus low molecular phenolic compounds (*e.g.* flavones, flavonols, flavonol glycosides, etc.) and therefore, the AH activity of CT-plants extract or fraction might be influenced by these phenolics or possible occurrence of interactions.

In contrast, for *T. colubriformis*, PD/PC ratio was consistently (*e.g.* all sort of fractions, F1 and/or F2) related to AH activity. This agrees with other reports on *T. colubriformis* larvae, which found higher AH *in vitro* effects of PD-rich tannins compared with PC-rich tannins (Brunet and Hoste, 2006; Molan et al., 2003; Molan et al., 2004).

2. *In vivo* studies:

For the *in vivo* studies the objectives were to compare the effects of sainfoin pellets vs hazelnut skin [which possess either PD rich (sainfoin) or PC rich (hazelnut) tannin structure] on: i) the biology of one abomasal and one intestinal nematodes species in lambs, ii) the host's resilience (Article 3), and iii) to evaluate the possible variations of CTs in their transit along the digestive tract regarding to: quantity (concentration) and/or quality (structure) in order to evaluate if such variations might be related to the *in vivo* AH activity and the differences between species (Article 4).

Some *in vivo* studies have been previously conducted in small ruminants to evaluate AH effect of the by-products (*i.e.* hazelnut skin) either separately (Desrues et al., 2012) or in combination with sainfoin (Girard et al., 2013). So far, to the best of our knowledge, our studies (Article 3 and Article 4) are the first aimed at comparing the AH effect of by-products with CT forage (*i.e.* sainfoin) in the same experiment and at relating this activity with the concentration, structure and reactivities of tannin in a range of digesta samples and faeces (Article 4).

In order to mainly evaluate the role of CT's structural features (*i.e.* quality) on the *in vivo* AH activity, the 2 resources were chosen because of high differences in their PD/PC ratios. According to the findings mentioned in the Article 1, the PD/PC ratios were respectively 75/25 for sainfoin pellets whereas 28/72 for hazelnut skin. Instead, the mDP values in both resources were similar, 11.5 for sainfoin pellets and 13.3 for hazelnut skin. In addition, the experimental protocol was designed in order to limit as much as possible the differences in CT quantity between the lambs receiving either sainfoin or hazelnut skins. This was initially based on calculation in the diet but did not take into account the amount of refusals by the lambs. However, based on the results presented in Article 4, it seems that, in regard of tannin contents in the different digestive organs, similar CT values have been achieved in both groups.

- **Effects of two CT-containing resources differing in quality (PD/PC ratio) in the parasite's biology:**

To summarize the results obtained in this study (Article 3), the main AH effects observed in the lambs consuming sainfoin pellets (SG) were a consistent reduction of FEC, reduction in EPG of *H. contortus*, a trend for lower total burden of *H. contortus* compared with the control and HG group. On the other hand, the group consuming hazelnut skin (HG) as CT-source, showed a reduction on female fecundity for *T. colubriformis*, which explain the specific reductions of FEC observed for this species during the trial and the reduction of *T. colubriformis* proportion in the larvae cultures.

Several previous studies have reported a decrease in FEC in small ruminants when fed with sainfoin regardless whether exploited as fresh (Athanasiadou et al., 2005; Paolini et al., 2005), silage (Heckendorn et al., 2006), hay (Heckendorn et al., 2006) or pellets (Girard et al., 2013; Werne et al., 2013).

Studies to examine the AH effect of hazelnut skin are far less abundant. Girard et al. (2013) observed trends in reduction of FEC in lambs infected with *H. contortus* when hazelnut skin was added to a sainfoin pellets ration and Desrues et al. (2012) recorded significant reductions of FEC in goats infected with *H. contortus* and *T.colubriformis* and fed with hazelnut skin as CT source. These differences with our current results might be related with the amount of hazelnut skin offered, which was higher in the previous studies than in ours, for instance 500 g/goat/day (Desrues et al., 2012) or 300 g/lamb/day (Girard et al., 2013). Another issue to take into account is either the host species (sheep/goat) or their different adaptations to cope with tannin diets, as increasing their tannin-binding salivary protein could affect the tannin avalaibility and metabolism in the digestive tract (Alonso-Díaz et al., 2012). Finally, in the results observed by Girard et al. (2013) some synergistic or additive effects might have occurred between the CT from the sainfoin pellets offered as a basal diet and those from the hazelnut skin added from D35 to D42.

In our results, with the consumption of hazelnut skin no significant reduction in worm population was observed neither for *H. contortus* nor *T. colubriformis*. This lack of effect agrees with previous findings described in goats (Desrues et al., 2012) and sheep (Girard et al., 2013).

Based on the hypothesis that similar quantity of CT content has been ingested by the lambs in the SG and HG groups, these results tend to support the hypothesis that the quality of CT can also influence the AH effects in *in vivo* conditions since the consumption of the resource with the higher PD/PC values (sainfoin) was associated with some signs of AH activity. This statement is in agreement with our *in vitro* results (Articles 1 and 2).

- **Effects of two CT-containing resources differing in quality (PD/PC ratio) in the host's resilience:**

The PCV (packed cell volume) was measured to evaluate the severity of lamb's anemia and was used as an indicator of the host resilience status (Article 3). In the SG animals, some positive effect on host resilience was observed as measured by the PCV values and a trend ($P < 0.10$) for higher PCV during the period of full CT-rations consumption (*i.e.* from D28 up to D55). The date by date analyses confirm, on 2 dates, PCV values significantly higher in the SG than in the HG or CG animals. This improvement in hematocrit values had been reported in either goats (Paolini et al., 2005) or sheep consuming sainfoin (Arroyo-Lopez et al., 2014; Azuhnwi et al., 2013) but also for hazelnut diet (Desrues et al., 2012; Girard et al., 2013). These effects on host resilience are important for the lambs survival and production (Urquhart et al., 1996).

Further studies including other pathophysiological parameters or production measurements are required to evaluate the actual impact of CTs quality on the host resilience.

- **Tannin content and reactivities of two CT-containing resources differing in quality (PD/PC ratio) in the digestive tract of sheep:**

Two hypotheses have been emitted to explain the variability depending on the nematode species. The possibility that this is related to some intrinsic features, related to protein composition of some anatomical parts of the different nematode stages has been discussed above in the section "CT *in vitro* AH effect per parasite species"**.**

A second hypothesis is that the difference in AH effects between nematode (in particular, between abomasal vs intestinal) species mentioned in the literature and summarised in the Table 3 of the article submitted to *Adv. Parasit*. could be explained by differences of exposition to bioavailable CTs in terms of quantity and quality depending on the digestive organs and related environmental conditions for the worms.

So far, very few studies have been conducted in order to evaluate the availability of CT along the digestive tract of small ruminants, and they were performed with a standard HCl-butanol assay (Perez-Maldonado and Norton, 1996; Terrill et al., 1994). Moreover, these studies did not include any parasite infections. The protocol used in our study (with an improved HCl-butanol method) allows to extract and measure CT by up to 3.2-fold over the standard method run without acetone and ferric ion (Grabber et al., 2013). In addition, the use of a second method, the thiolysis assay with benzyl mercaptan (BM), gave information on the CT content and also on their structural features in terms of polymer size (mDP), PC/PD and *cis*/*trans* ratios (Gea et al., 2011). To our knowledge, this is the first study combining these 2 methods of CT measurements with two contrasting tannin containing resources in GIN infected sheep. A similar study has been perfomed in parallel in infected cattle fed with sainfoin (Desrues et al., 2015).

To summarize the main results:

- Altogether, our results on the CT values from lamb digesta samples, were lower than the CT-resources feed [*i.e.* sainfoin pellets (SG) or hazelnut skin (HG)]. in both experimental/diet groups, depending on the digestive organs, only 20 to 40 % (HG) and 15 to 33 % (SG) of the initial feed content were recovered in the digesta with the butanol HCl method. With the thiolysis, the values ranged from 7 to 11 % for the hazelnut skin group and 15 to 42 % for the SG lambs. In contrast to the butanol HCl method, it has been mentioned that CTs had to be free to react with the benzyl mercaptan in the thiolysis (Ramsay et al., 2015). The thiolysis method is specific to tannins. Hence, it is suspected that the CT values based on thiolysis indicate the amount of free extractable tannins available.

- Overall, the CT measurements in the samples of lambs from both SG and HG (Article 4), regardless of the method applied, showed the same pattern in the different compartment (rumen, abomasum, small intestine, faeces). For both groups of diet, the highest CT contents were observed in the abomasal digesta samples. However, with both methods and between both diets, no differences for the CT content values were found along the gut.

- Conversely, the previous numbers indicate high rates (over 50% of the initial feed content) of disappearance of CT along the gut. Similar level of losses have been reported by Perez-Maldonado and Norton (1996) and Terrill et al. (1994) for small ruminants. As mentioned in the Article 4, due the free CT are relatively large polymers to be absorbed, their apparent disappearance in digesta samples, might be related to different mechanisms like: i) degradation and subsequent flavanol monomer units absorption and/or ii) conformational changes in the CT structure, and/or iii) binding to protein or other macromolecules (Choy et al., 2014; Gladine et al., 2007; López-Andrés et al., 2013; Perez-Maldonado and Norton, 1996; Terrill et al., 1994).

As is explained by Mueller-Harvey, (2006) the CT bind proteins in close to neutral pH conditions, thus CT-protein complexes may be formed in rumen and then under the acid conditions in the abomasum (pH < 3.5) or alkaline in the intestine (pH > 7) be released (Terrill et al., 1994). Indeed, the CT content values obtained in our study agree with these reactivities.

- last, in regard of CT content, it is worth to underline that despite differences in the quantity distributed to the animals (ratio close to 5.5 between the sainfoin and the hazelnut quantities in the diet) the CT content recovered in the different gut segments did not differ statistically between diet groups, whatever the method of measurements.

- On the other hand, in regard of CT quality or structure (Article 4), the CT structural characteristics of each diet (*i.e.* mDP, PD/PC ratio) seemed mainly preserved in the set of samples from both groups, with the exception of an increase in mDP in digesta samples of SG. As the mDP values were preserved, it seems unlikely that CT have undergone just a depolymerization process by microbiota (Perez-Maldonado and Norton, 1996). Therefore, another degradation pathway might likely occur, direct degradation to phenolic acids (Appeldoorn et al., 2009) or spontaneous oxidation (Hagerman, 2012).

In the terminal and extension CT flavanol units, some compositional changes were observed in both experimental groups. For instance, in the SG samples in the terminal units no GC nor EGC were detected, whereas in the HG in few samples just traces of galloylated flavanols were observed, may be because of their higher binding capacity (Le Bourvellec et al., 2005). These facts suggest that the CT had undergone some changes during their transit along the digestive tract, irrespective of the quality (*i.e.* high or low PD/PC ratio).

The CT content measured by thiolysis seems to indicate the presence of free tannins in the digest all along the gut, in organs which are the site of key events of the nematode life cycle depending on the species [*i.e.* infective larvae (L3), larval exsheathment in the rumen and/or abomasum; main site of infection for adult worm populations in the abomasum and small intestine)] and eggs in the faeces. It has been suggested that the CTs protein-binding property accounts for the AH effects observed in CTs studies. Some external aggregates and/or surface and internal lesions have been observed in *in vivo* and *in vitro* studies with GIN adults (Martínez-Ortíz-de-Montellano et al., 2013) or larvae (Brunet et al., 2007) which have been exposed to CTs extracts. These observations, could seriously affect the nematode physiology. The nematode cuticle, but also the products of secretion/excretion (*i.e.* with main enzymatic activities) are protein-rich (Page, 2001). It has been hypothesized that the higher protein binding capacity of CTs, the better AH effect they may exert. The higher protein-binding flavan-3-ols EGCG and GCG showed *in vitro* the stronger AH effect for *H. contortus* and especially for *T. colubriformis* (Brunet and Hoste, 2006). Both monomers are prodelphinidins (PD), and the galloylated flavan-3-ols are pointed as more efficient in precipitate proteins (Hagerman, 2012). The mDP of CTs also accounts for protein precipitation (Stojadinovic et al., 2013). Both (*i.e.* PD/PC ratio and mDP) structural features have recently been reported as a key variable for the *in vitro* AH effect against parasites of cattle (Novobilský et al., 2013), pigs (Williams et al., 2014) and small ruminants (Articles 1 and 2).

Our results from the analyses of CT in digesta (Article 4) can be compared with the parasitological results exposed in Article 3 obtained in infected lambs fed with isotannic (same quantity of CT) diets which differ in their quality (*i.e.* PD/PC ratio) eventually. Similar CT content have been achieved in the different digestive organs. Some changes in the mDP values for the sainfoin and in the presence of galloylated flavanol in the HG groups were noticed. However, overall the PD/PC ratios of the two discriminant diets were conserved. Hence, it seems that some structural features (quality) contribute to the AH properties of tannins and the deleterious effects against parasites since the diet with the higher PD/PC ratio (sainfoin), showed more consistent AH effect *in vivo* despite similar CT concentration in the digesta. These preliminary results are consistent with previous *in vitro* findings (Article 1 and 2).

CONCLUSIONS et PERSPECTIVES

Ce travail de thèse a eu pour objectif général de mieux comprendre les relations entre la structure des tanins condensés (TCs) et leur activité contre les nématodes gastro-intestinaux (NGIs) des petits ruminants, ceci afin de mieux identifier l'origine des variabilités de résultats observés.

Des données antérieures avaient montré la complexité des interactions existantes entre les tanins et les nématodes gastro-intestinaux. Les relations dose–réponse ont été en premier lieu explorées, à la fois en conditions *in vitro* et *in vivo* et les résultats acquis laissent supposer qu'un seuil minimal de TCs dans la ration est nécessaire [> 3 à 4 % de la MS (Hoste et al. 2006 ; Mueller-Harvey 2006)], pour obtenir un effet AH significatif. Par ailleurs, des premières données ont été obtenues indiquant que la nature biochimique des TCs était aussi un facteur important modulant les effets anthelminthiques (AH). Des plantes contenant des TCs présentant un ratio prodelphinidol/procyanidol (PD/PC) élevé ont été décrites comme plus actives contre les vers parasites (Molan et al., 2003, 2004). Enfin, certaines études ont montré qu'à côté des TCs, certaines molécules flavonoïdes simples présentaient aussi des effets AHs (Molan et al., 2004). Ainsi les monomères flavan-3-ols constitutifs des tannins de type PD ont montré une plus forte activité antiparasitaire que ceux composant les procyanidols (Brunet et Hoste, 2006).

Au travers des études *in vitro* de notre thèse, nous avons confirmé que le ratio PD/PC mais aussi la taille (mDP) des TCs influencent les activités AHs. Ces résultats confirment ceux obtenus sur des nématodes parasites de porcs (Williams et al., 2014) et de bovins (Novobilský et al., 2013). De manière générale, *H. contortus* s'est avéré plus sensible (valeurs EC50 plus faibles) aux effets des diverses fractions que *T. colubriformis* mais nos résultats ont souligné des différences de réponse spécifique en fonction de la qualité des tannins. De plus, nos études *in vitro* ont aussi souligné la complexité des interactions entre TCs et NGIs, puisque des synergies d'effets AH entre tanins et flavonoïdes (quercétine et lutéoline) ont été relevées tout particulièrement dans le cas des TCs avec un faible rapport PD/PC.

Les résultats de l'étude *in vivo* tendent à confirmer le rôle de facteurs qualitatifs (ratio PD/PC) chez les animaux, des effets anthelminthiques plus nets et une meilleure résilience ayant été observé chez les agneaux consommant du sainfoin (*Onobrychis viciifolia), (*avec un ratio PD/PC élevé) alors que les effets chez ceux recevant des coproduits de la noisette (*Corylus avellana)* avec un ratio PD/PC bas ont été beaucoup plus discrets. Enfin, les analyses menées sur les contenus des divers organes digestifs et les fèces, pour les deux types de ressources, sont parmi les premières visant à explorer la « pharmacologie » des TC le long du tube digestif afin d'en analyser la biodisponibilité qui gouverne l'exposition des nématodes aux polyphénols et à leurs possibles propriétés AH. Les résultats ont montré de fortes réductions de TCs détectés par rapport aux teneurs initiales dans la ration, et à l'inverse une préservation globale des caractéristiques de structure au long du tube digestif. Ces derniers résultats viennent conforter l'hypothèse du rôle de la structure des TCs *in vivo*.

Le rôle de la qualité des TCs dans l'activité AH semble donc confirmé à la fois par nos données *in vitro* et *in vivo.* Ces résultats montrent l'importance de prendre en compte ces facteurs dans la recherche de ressources pouvant être exploitées comme futurs nutricaments chez les petits ruminants (Hoste el at., 2015).

Au vu des résultats obtenus pendant cette thèse, il parait nécessaire de continuer les études sur les relations structure/activité des tanins afin de mieux comprendre les mécanismes d'action, notamment par rapport à leur métabolisme et leur distribution dans les divers organes digestifs. De telles études fondamentales permettront d'orienter le choix de l'espèce végétale et la forme (technologie) de distribution des plantes bioactives pour exploiter ces ressources comme nutricaments dans une approche intégrée de la maîtrise des nématodes GIs chez les petits ruminants.

REFERENCES

Ademola, I.O., Fagbemi, B.O., Idowu, S.O., 2007. Anthelmintic activity of *Spigelia anthelmia* extract against gastrointestinal nematodes of sheep. *Parasitol.* Res., 101, 63–69.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Hoste, H., 2010. Tannins in tropical tree fodders fed to small ruminants: A friendly foe? *Small Ruminant Res.,* 89, 164–173.

Alonso-Díaz, M.A., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Capetillo-Leal, C.M., 2012. Amino acid profile of the protein from whole saliva of goats and sheep and its interaction with tannic acid and tannins extracted from the fodder of tropical plants. *Small Ruminant Res.,* 103, 69–74.

Andronicos, N., Hunt, P., Windon, R., 2010. Expression of genes in gastrointestinal and lymphatic tissues during parasite infection in sheep genetically resistant or susceptible to *Trichostrongylus colubriformis* and *Haemonchus contortus*. *Int. J. Parasitol*., 40, 417–29.

Appeldoorn, M.M., Vincken, J.P., Aura, A. M., Hollman, P.C., Gruppen, H., 2009. Procyanidin dimers are metabolized by human microbiota with 2-(3,4-dihydroxyphenyl) acetic acid and 5-(3,4-dihydroxyphenyl)-gamma-valerolactone as the major metabolites. *J. Agric. Food Chem.,* 57, 1084–1092.

Arroyo-Lopez, C., Manolaraki, F., Saratsis, A., Saratsi, K., Stefanakis, A., Skampardonis, V., Voutzourakis, N., Hoste, H., Sotiraki, S., 2014. Anthelmintic effect of carob pods and sainfoin hay when fed to lambs after experimental trickle infections with *Haemonchus contortus* and *Trichostrongylus colubriformis*. *Parasite,* 21, 71.

Assis, R.C.L., Luns, F.D., Araújo, J. V, Braga, F.R., 2012. Biological control of trichostrongyles in beef cattle by the nematophagous fungus *Duddingtonia flagrans* in tropical southeastern Brazil. *Exp. Parasitol.,* 132, 373–7.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2000. Effects of short-term exposure to condensed tannins on adult *Trichostrongylus colubriformis*. *Vet. Rec.,* 146, 728-732.

Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2001. Direct anthelmintic effects of condensed tannins towards different gastrointestinal nematodes of sheep: *in vitro* and *in vivo* studies. *Vet. Parasitol.,* 99, 205-219.

Athanasiadou, S., Tzamaloukas, O., Kyriazakis, I., Jackson, F., Coop, R.L., 2005. Testing for direct anthelmintic effects of bioactive forages against *Trichostrongylus colubriformis* in grazing sheep. *Vet. Parasitol.,* 127, 233–243.

Athanasiadou, S., Houdijk, J., Kyriazakis, I., 2008. Exploiting synergisms and interactions in the nutritional approaches to parasite control in sheep production systems. *Small Ruminant Res.,* 76, 2–11.

Ayres, M.P., Clausen, P.H., Maclean, S.F., Redman, A.M., Reichart, P.B., 1997. Diversity of structure and antiherbivore activity in condensed tannins. *Ecology*, 78, 1696-1712.

Azuhnwi, B.N., Hertzberg, H., Arrigo, Y., Gutzwiller, A., Hess, H.D., Mueller-Harvey, I., Torgerson, P.R., Kreuzer, M., Dohme-Meier, F., 2013. Investigation of sainfoin (*Onobrychis viciifolia*) cultivar differences on nitrogen balance and fecal egg count in artificially infected lambs. *J. Anim. Sci*., 91, 2343–2354.

Bahuaud, D., Martinez-Ortiz De Montellano, C., Chauveau, S., Prevot, F., Torres-Acosta, F., Fouraste, I., Hoste, H., 2006. Effects of four tanniferous plant extracts on the *in vitro* exsheathment of third-stage larvae of parasitic nematodes. *Parasitology*, 2006, 132, 545-554.

Baker, R.L., Mwamachi, D.M., Audho, J.O., Aduda, E.O., Thorpe, W., 1998. Resistance of Galla and Small East African goats in the sub-humid tropics to gastrointestinal nematode infections and the peri-parturient rise in faecal egg counts. *Vet. Parasitol*., 79, 53-64.

Balic, A., Bowles, V.M., Meeusen, E.N., 2000. The immunobiology of gastrointestinal nematode infections in ruminants. *Adv. Parasit*., 45, 181-241.

Balic, A., Bowles, V.M., Meeusen, E.N.T., 2002. Mechanisms of immunity to *Haemonchus contortus* infection in sheep. *Parasite Immunol.,* 24, 39–46.

Bambou, J.-C., de la Chevrotière, C., Varo, H., Arquet, R., Kooyman, F.N.J., Mandonnet, N., 2008. Serum antibody responses in Creole kids experimentally infected with *Haemonchus contortus*. *Vet. Parasitol*., 158, 311–8.

Barger, I.A., 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *Int. J. Parasitol.,* 29, 41-47.

Barrau, E., Fabre, N., Fouraste, I., Hoste, H., 2005. Effect of bioactive compounds from Sainfoin (*Onobrychis viciifolia* Scop.) on the *in vitro* larval migration of *Haemonchus contortus*: role of tannins and flavonol glycosides. *Parasitology*, *131*, 531-538.

Bartley, D.J., Devin, L., Nath, M., Morrison, A. A., 2015. Selection and characterisation of monepantel resistance in *Teladorsagia circumcincta* isolates. *Int. J. Parasitol. Drugs Drug Resist.*, 5, 69–76.

Bassetto, C.C., Picharillo, M.É., Newlands, G.F.J., Smith, W.D., Fernandes, S., Siqueira, E.R., Amarante, A.F.T., 2014a. Attempts to vaccinate ewes and their lambs against natural infection with *Haemonchus contortus* in a tropical environment. *Int. J. Parasitol.,* 44, 1049–54.

Bassetto, C.C., Silva, M.R.L., Newlands, G.F.J., Smith, W.D., Ratti Júnior, J., Martins, C.L., Amarante, A.F.T., 2014b. Vaccination of grazing calves with antigens from the intestinal membranes of *Haemonchus contortus*: effects against natural challenge with *Haemonchus placei* and *Haemonchus similis*. *Int. J. Parasitol*., 44, 697–702.

Bate-Smith, E. 1973. Tannins of herbaceous leguminosae. *Phytochemistry*, 12, 1809-1812.

Baxter, N.J., Lilley, T.H., Haslam, E., Williamson, M.P., 1997. Multiple interactions between polyphenols and a salivary proline-rich protein repeat result in complexation and precipitation. *Biochemistry*, 36, 5566-5577.

Bengone-Ndong, T., Alvinerie, M., 2004. Macrolides antiparasitaires : propriétés pharmacologiques générales et recommandations d'usage dans le contexte vétérinaire africain. *Revue Elev. Méd. Vét. Pays Trop*., 57, 49-58.

Bennick, A., 2002. Interaction of plant polyphenols with salivary proteins. *Crit. Rev. Oral Biol. Med.,* 13, 184-96.

Besier, B., 2007. New anthelmintics for livestock: the time is right. *Trends Parasitol.*, 23, 21-24.

Beugnet, F., Gevrey, J., Kerboeuf, D., 1997. Les endectocides: mode d'action et utilisation. *Point Vét.*, 28, 1915-1919.

Beynon, S.A., 2012. Potential environmental consequences of administration of anthelmintics to sheep. *Vet. Parasitol.,* 189, 113–24.

Bishop, S.C. and Morris, C.A., 2007. Genetics of disease resistance in sheep and goats. *Small Ruminant Res.,* 70, 48-59.

Bliss, C.I., 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol.,* 26, 585-615.

Borreani, G., Peiretti, P.G., Tabacco, E., 2003. Evolution of yield and quality of sainfoin (*Onobrychis viciifolia* scop.) in the spring growth cycle. *Agronom*., 23, 193- 201.

Bricarello, P.A., Amarante, A.F.T., Rocha, R.A., Cabral Filho, S.L., Huntley, J.F., Houdijk, J.G.M., Abdalla, A.L., Gennari, S.M., 2005. Influence of dietary protein supply on resistance to experimental infections with *Haemonchus contortus* in Ile de France and Santa Ines lambs. *Vet. Parasitol*., 134, 99–109.

Brunet, S., Hoste, H., 2006. Monomers of condensed tannins affect the larval exsheathment of parasitic nematodes of ruminants. *J. Agric. Food Chem*., 54, 7481–7.

Brunet, S., Aufrere, J., El-Babili, F., Fouraste, I., 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., Jackson, F., Hoste, H., 2008. Effects of sainfoin (*Onobrychis viciifolia*) extract and monomers of condensed tannins on the association of abomasal nematode larvae with fundic explants. *Int. J. Parasitol.,* 38, 783–790.

Brunet, S., Fourquaux, I., Hoste, H., 2011. Ultrastructural changes in the third-stage, infective larvae of ruminant nematodes treated with sainfoin (*Onobrychis viciifolia*) extract. *Parasitol. Int.,* 60, 419–424.

Bruneton, J., 1999. Tannins. In: Pharmacognosie, phytochimie, Plantes médicinales, TEC&DOC (Ed.), Paris, pp. 369-404.

Burke, J.M., Whitley, N.C., Pollard, D. A., Miller, J.E., Terrill, T.H., Moulton, K.E., Mosjidis, J. A., 2011. Dose titration of sericea lespedeza leaf meal on *Haemonchus contortus* infection in lambs and kids. *Vet. Parasitol.,* 181, 345–9.

Burke, J.M., Miller, J.E., Mosjidis, J. A., Terrill, T.H., 2012. Grazing sericea lespedeza for control of gastrointestinal nematodes in lambs. *Vet. Parasitol*., 186, 507–12.

Butter, N.L., Dawson, J.M., Buttery, P.J., 1999. Effects of dietary tannins on ruminants. In: Secondary Plant Products. Nottingham-University-Press, Nottingham, pp. 51-70.

Cadot, Y., Miñana Castelló, M.T.C., M., 2006. Flavan-3-ol compositional changes in grape berries (*Vitis vinifera* L. cv Cabernet Franc) before veraison, using two complementary analytical approaches, HPLC reversed phase and histochemistry. *Anal. Chim. Acta*, 563, 65-75.

Cala, O., Dufourc, E.J., Fouquet, E., Manigand, C., Laguerre, M., Pianet, I., 2012. The colloidal state of tannins impacts the nature of their interaction with proteins: The case of salivary proline-rich protein/procyanidins binding. *Langmuir*, 28, 17410–17418.

Carlsen, S.C.K., Fomsgaard, I.S., 2008. Biologically active secondary metabolites in white clover (*Trifolium repens* L.) - A review focusing on contents in the plant, plant-pest interactions and transformation. *Chemoecology*, 18, 129-170.

Cenci, F.B, Louvandini, H., McManus, C.M., Dell'Porto, A., Costa, D.M., Arau´jo, S.C., Minho, A.P., Abdalla, A.L., 2007. Effects of condensed tannin from *Acacia mearnsii* on sheep infected naturally with gastrointestinal helminthes. *Vet. Parasitol.*, 144, 132-137.

Chandrawathani, P., Adnan, M., Waller, P.J., 1999. Anthelmintic resistance in sheep and goat farms on Peninsular Malaysia. *Vet. Parasitol.,* 82, 305-310.

Charlier, J., Morgan, E.R. , Rinaldi, van Dijk, J., Demeler, J., Hoglund, J., Hertzberg, H., Van Ranst, B., Hendrickx, G., Vercruysse, J., 2014. Practices to optimise gastrointestinal nematode control on sheep, goat and cattle farms in Europe using targeted (selective) treatments. *Vet Rec.*, 175, 250-255.

Chartier, C., Reche, B., 1992. Gastrointestinal helminths and lungworms of french dairy goats: prevalence and geographical distribution in Poitou-Charentes. *Vet. Res. Com.*, 16, 327-335.

Chartier, C., Hoste, H., 1994. Anthelmintic treatments against digestive-tract nematodes in grazing dairy goats with high or low levels of milk production. *Vet. Parasitol.* 25, 450-457.

Chartier, C., Itard, J., Morel, P., Troncy, P., 2000. Précis de parasitologie vétérinaire tropicale, Paris.

Cheynier V., Souquet J.-M., Roux E.L., Guyot S., Rigaud J., 1999. Size separation of condensed tanins by normal-phase high-performance liquid chromatography. *Methods in enzymol.*, 299, 178-184.

Choy, Y.Y., Quifer-Rada, P., Holstege, D.M., Frese, S. a., Calvert, C.C., Mills, D. a., Lamuela-Raventos, R.M., Waterhouse, A.L., 2014. Phenolic metabolites and substantial microbiome changes in pig feces by ingesting grape seed proanthocyanidins. *Food Funct.*, 5, 2298–2308.

Coblentz, W.K., Grabber, J.H., 2013. In situ protein degradation of alfalfa and birdsfoot trefoil hays and silages as influenced by condensed tannin concentration. *J. Dairy Sci.,* 96, 3120–3137.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M. A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.*, 44, 35–44.

Coop, R.L., Kyriazakis, I., 2001. Influence of host nutrition on the development and consequences of nematode parasitism in ruminants. *Trends Parasitol*., 17, 325-330.

Costa-Júnior, L., Gracione Sousa-Filho, J., Sousa-Junior, A., Traesel, L., Santos, A., Sousa, Hyanne Lima, M., Almeida, M., 2015. Failure of anthelmintic treatment with monepantel in sheep in Brazil. Proceedings: 25th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP). 16-20 August, Liverpool, UK, 2015. p 560.

Cotter, J.L., Van Burgel, A., Besier, R.B., 2015. Anthelmintic resistance in nematodes of beef cattle in south-west Western Australia. *Vet. Parasitol.*, 207, 276-284.

Cruz, D.G., Costa, L.M., Rocha, L.O., Retamal, C.A., Vieira, R.A.M., Seabra, S.H., Silva, C.P., DaMatta, R.A., Santos, C.P., 2015. Serine proteases activity is important for the interaction of nematophagous fungus *Duddingtonia flagrans* with infective larvae of trichostrongylides and free-living nematodes *Panagrellus* spp. *Fungal Biol*., 119, 672–8.

Dawson, L.E.R., McCoy, M. A., Edgar, H.W.J., Carson, A. F., 2011. Effect of concentrate supplementation at pasture and inclusion of condensed tannins (Quebracho) in concentrates on lamb performance and faecal egg and worm counts. *Livest. Sci*., 135, 205–214.

De Rosa, A.A., Chirgwin, S.R., Fletcher, J., Williams, J.C., Klei, T.R., 2005. Exsheathment of *Ostertagia ostertagi* infective larvae following exposure to bovine rumen contents derived from low and high roughage diets. *Vet. Parasitol.*, 129, 77–81.

Desrues, O., Vargas-Magaña, J.J., Girard, M., Manolaraki, F., Pardo, E., Mathieu, C., Vilarem, G., Torres-Acosta, J.F.J., Sandoval-Castro, C.A., Jean, H., Hoste H., 2012. Can hazel-nut peels be used to control gastrointestinal nematodes in goats? XIth International Goat Conference, Las Canarias, Sept 2012.

Desrues, O., Peña-Espinoza, M., Hansen, T.V., Mueller-Harvey, I., Enemark, H.L., Thamsborg, S.M., 2015. Anthelmintic effects of sainfoin against different cattle nematodes may be linked to concentration of condensed tannins in different gut compartments. 25th WAAVP conference. Liverpool, UK, August 2015.

Diaz Lira, C.M., Barry, T.N., Pomroy, W.E., McWilliam, E.L., Lopez-Villalobos, N., 2008. Willow (*Salix* spp.) fodder blocks for growth and sustainable management of internal parasites in grazing lambs. *Anim. Feed Sci. Technol.*, 141, 61–81.

Dobreva, M.A., Green, R.J., Mueller-harvey, I., Salminen, J., Howlin, B.J., Frazier, R.A., 2014. Size and molecular flexibility affect the binding of ellagitannins to bovine serum albumin. *J. Agric. Food Chem.*, 62, 9186−9194.

Douch, P.G., Green, R.S., Morris, C.A., Mceewan, J.C., Windon, R.G., 1996. Phenotypic markers for selection of nematode-resistant sheep. *Int. J. Parasitol.,* 26, 899-911.

Etter, E., Chartier, C., Hoste, H., Pors, I., Lefrileux, Y., Broqua, C., Vallade, S. et Goudeau, C., 2000. Parasitisme par les nématodes du tube digestif et utilisation du pâturage: Epidémiologie de l'infestation dans les troupeaux caprins laitiers en France. *Epidémio. Santé Anim.,* 37, 75-86.

Falzon, L.C., Menzies, P.I., Shakya, K.P., Jones-Bitton, A., Vanleeuwen, J., Avula, J., Jansen, J.T., Peregrine, A.S., 2013. A longitudinal study on the effect of lambing season on the periparturient egg rise in Ontario sheep flocks. *Prev. Vet. Med*., 110, 467–80.

Feucht, W., Treutter, D., Christ, E., 1997. Role of flavanols in yellowing beech trees of the Black Forest. *Tree Physiology*, 17, 335–340.

Foo, L.Y., Newman, R., Waghorn, G., Mc Nabb, W.C., Ulyatt, M.J., 1996. Proanthocyanidins from *Lotus corniculatus*. *Phytochemistry,* 41, 617-624.

Foo, L.Y., Lu, Y., Mc Nabb, W.C., Waghorn, G., Ulyatt, M.J., 1997. Proanthocyanidins from *Lotus pedunculatus*. *Phytochemistry,* 45, 1689-1696.

Frutos, P., Hervas, G., Ramos, G., Giraldez, F.J., Mantecon, A.R., 2002. Condensed tannin content of several shrub species from a mountain area in northern Spain, and its relationship to various indicators of nutritive value. *Anim. Feed Sci. Technol*., 95, 215-226.

Gamble, H.R., Lichtenfield, J.R., Purcell, J.P., 1989. Light and scanning electron microscopy of the ecdysis of *Haemonchus contortus* infective larvae. *J. Parasitol.*, 75,303-307.

Gauly, M., Erhardt, G., 2001. Genetic resistance to gastrointestinal nematode parasites in Rhön sheep following natural infection. *Vet. Parasitol.,* 102, 253-259.

Gea, A., Stringano, E., Brown, R.H., Mueller-Harvey, I., 2011. *In situ* analysis and structural elucidation of sainfoin (*Onobrychis viciifolia*) tannins for high-throughput germplasm screening. *J. Agric. Food Chem.,* 59, 495–503.

George, S.D., George, A.J., Stein, P.A., Rolfe, P.F., Hosking, B.C., Seewald, W., 2012. The comparative efficacy of abamectin, monepantel and an abamectin/derquantel combination against fourth-stage larvae of a macrocyclic lactone-resistant *Teladorsagia* spp. isolate infecting sheep. *Vet. Parasitol.*, 188, 190–3.

Girard M., Gaid S., Mathieu C., Vilarem G., Gerfault V., Routier M., Gombault P., Pardo E., Manolaraki F., Hoste H., 2013. Effects of different proportions of sainfoin pellets combined with hazel nut peels on infected lambs. 64th EAAP, Nantes, 26th-30th August, 2013.

Githiori, J.B., Athanasiadou, S., Thamsborg, S.M., 2006. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants. *Vet. Parasitol.*, 139, 308–320.

Gladine, C., Rock, E., Morand, C., Bauchart, D., Durand, D., 2007. Bioavailability and antioxidant capacity of plant extracts rich in polyphenols, given as a single acute dose, in sheep made highly susceptible to lipoperoxidation. *Br. J. Nutr.*, 98, 691–701.

Grabber, J.H., Zeller, W.E., Mueller-Harvey, I., 2013. Acetone enhances the direct analysis of procyanidin- and prodelphinidin-based condensed tannins in lotus species by the butanol-HCl-iron assay. *J. Agric. Food Chem.*, 61, 2669–2678.

Gray, G.D., 1997. The use of genetically resistant sheep to control nematode parasitism. *Vet. Parasitol.,* 72, 345-366.

Gruner, L., Bouix, J., Vu Tien Khang, J., 1998. La résistance génétique aux nématodes parasites chez les petits ruminants : un caractère de mieux en meiux connu. *Renc. Rech. Rum*., 8, 195-198.

Guedes, M.T., Zacharias, F., Couto, R.D., Portela, R.W., Santos, L.C.S., Santos, S.C.O., Pedroza, K.C., Peixoto, A.P.C., López, J.A., Mendonça-Lima, F.W., 2010. Maternal transference of passive humoral immunity to *Haemonchus contortus* in goats. *Vet. Immunol. Immunopathol.*, 136, 138–143.

Hagerman, A.E., 2002. Tannin Chemistry (www.users.muohio.edu/hagermae).

Hagerman, A.E., 2012. Fifty years of polyphenol-protein complexes. In: Recent advances in polyphenol research. 1st Edition. Cheynier, V.; Sami-Machado, P.; Quideau, S. Eds. Publisher: Wiley-Blackwell & sons, Oxford, UK. Vol. No. 3. 364 pp.

Haile, A., Tibbo, M., Baker, R.L., Rege, J.E.O., 2007. Effects of non-genetic factors on responses to gastro-intestinal nematode infections in Ethiopian sheep. *Trop. Anim. Health Prod.,* 39, 411-417.

Heckendorn, F., Häring, D.A., Maurer, V., Zinsstag, J., Langhans, W., Hertzberg, H., 2006. Effect of sainfoin (*Onobrychis viciifolia*) silage and hay on established populations of *Haemonchus contortus* and *Cooperia curticei* in lambs. *Vet. Parasitol.*, 142, 293–300.

Heckendorn, F., 2007. The control of gastrointestinal sheep, nematodes with taniferous forage plants. Swiss Federal Institute of Technology, Zurich, Switerland

Hedqvist, H., Mueller-Harvey, I., Reed, J.D., Krueger, C.G., Murphy, M., 2000. Characterization of tannins and *in vitro* protein digestibility of several *Lotus corniculatus* varieties. *Anim. Feed Sci. Technol.,* 87, 41-56.

Hertzberg, H., Huwyler, U., Kohler, L., Rehbein, S., Wanner, M., 2002. Kinetics of exsheathment of infective ovine and bovine strongylid larvae *in vivo* and *in vitro*. *Parasitology,* 125, 65-70.

Hoskin, S.O., Wilson, P.R., Barry, T.N., Charleston, W.A., Waghorn, G.C., 2000. Effect of forage legumes containing condensed tannins on lungworm (*Dictyocaulus* sp.) and gastrointestinal parasitism in young red deer (*Cervus elaphus*). *Res. Vet. Sci.,* 68, 223-230.

Hoste, H., Chartier, C., 1993. Comparison of the effects on milk production of concurrent infection with *Haemonchus contortus* and *Trichostrongylus colubriformi*s in high- and low-producing dairy goats. *Am. J. Vet. Res.,* 54, 1886-1893.

Hoste, H., Huby, F., Mallet, S., 1997. Strongyloses gastro-intestinales des ruminants : conséquences physiopathologiuqes et mécanimses pathogéniques. *Point Vét.,* 28 (Parasitologie des ruminants), 53-59.

Hoste, H., Chartier, C., 1998. Response to challenge infection with *Haemonchus contortus* and *Trichostrongylus colubriformis* in dairy goats. Consequences on milk production. *Vet. Parasitol.,* 74, 43-54.

Hoste, H., Le Frileux, Y., Pommaret, A., Gruner, L., Van Quackebeke, E. et Koch, C., 1999. Importance du parasitisme par des strongles gastro-intestinaux chez les chèvres laitières dans le Sud-Est de la France. *INRA Prod. Anim.,* 12, 377-389.

Hoste, H., Leveque, H., Dorchies, P., 2001. Comparison of nematode infections of the gastrointestinal tract in Angora and dairy goats in a rangeland environment: relations with the feeding behaviour. *Vet. Parasitol.*, 101, 127-135.

Hoste, H., Chartier, C., Le Frileux, Y., Goudeau, C., Broqua, C., Pors, I., Bergeaud, J.P., Dorchies, P., 2002a. Distrubution and repeatability of nematode faecal egg counts in dairy goats: a farm survey and implications for worm control. *Res. Vet. Sci.*, 72, 211-215.

Hoste, H., Chartier, C., Le Frileux, Y., Goudeau, C., Broqua, C., Pors, I., Bergeaud, J.P., Dorchies, P., 2002b. Targeted application of anthelmintics to control trichostrongylosis in dairy goats: a result of a 2-year survey in farms. *Vet. Parasitol.*, 110, 101–108.

Hoste, H., Paolini, V., Paraud, C., Chartier, C., 2004. Gestion non-chimique du parasitisme par les nématodes chez les petits ruminants. *Bulletin G.T.V.* (Horssérie Parasitologie des ruminants laitiers), 131-135.

Hoste, H., Gaillard, H., Le Frileux, Y., 2005a. Consequences of the regular distribution of sainfoin hay on gastrointestinal parasitism with nematodes and milk production in dairy goats. *Small Ruminant Res.,* 59, 265-271.

Hoste, H., Torres-Acosta, F., Paolini, V., Aguilar-Caballero, A.J., Etter, E., Le Frileux, Y., Chartier, C., Broqua, C., 2005b. Interactions between nutrition and gastrointestinal infections with parasitic nematodes in goats. *Small Ruminant Res.,* 60, 141-151.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S., Hoskin, S.O., 2006. The effects of tannin-rich plants on parasitic nematodes in ruminants. *Trends Parasitol*., 22, 253-261.

Hoste, H., Sotiraki, S., Landau, S.Y., Jackson, F., Beveridge, I., 2010. Goat-nematode interactions: think differently. *Trends Parasitol.* 26, 376–81.

Hoste, H., Torres-Acosta, J.F.J., 2011. Non chemical control of helminths in ruminants: Adapting solutions for changing worms in a changing world. *Vet. Parasitol*., 180, 144–154.

Hoste, H., Martinez-Ortiz-De-Montellano, C., Manolaraki, F., Brunet, S., Ojeda-Robertos, N., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., 2012. Direct and indirect effects of bioactive tannin-rich tropical and temperate legumes against nematode infections. *Vet. Parasitol*., 186, 18–27.

Hoste, H., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Mueller-Harvey, I., Sotiraki, S., Louvandini, H., Thamsborg, S.M., Terrill, T.H., 2015. Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock. *Vet. Parasitol.,* 212, 5–17.

Hoste, H., Torres-Acosta, J.F.J., Quijada, J., Chan Perez, I., Dakheel, M.M., Kommuru, D.S, Mueller-Harvey, I., Terrill, T.H. Interactions between nutrition and infections with *H. contortus* (and related gastro intestinal nematodes) in small ruminants. *Adv. Parasit.* (In press).

Houdijk, J.G.M., 2012. Differential effects of protein and energy scarcity on resistance to nematode parasites. *Small Ruminant. Res.*, 103, 41–49.

Houdijk, J.G.M., Kyriazakis, I., Kidane, A., Athanasiadou, S., 2012. Manipulating small ruminant parasite epidemiology through the combination of nutritional strategies. *Vet. Parasitol*., 186, 38–50.

Hounzangbe-Adote, S., 2004. Propriétés anthelminthiques de 4 plantes tropicales testées *in vitro* et *in vivo* sur les nématodes gastro-intestinaux chez les petits ruminants Djallonke. Université d'Abomey-Calavi, Abomey-Calavi, Benin. 184 pp.

Hotez, P.J., Bethony, J., Bottazzi, M. E., Brooker, S., Diemert, D., Loukas, A., 2006. New technologies for the control of human hookworm infection. *Trends Parasitol.*, 22, 327-331.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003. Can animals use foraging behaviour to combat parasites ? *Proc. Nutr. Soc.,* 62, 361-370.

Jackson, F. et Miller, J., 2006. Alternative approaches to control: Quo vadit? *Vet. Parasitol.,* 139, 371-384

Jackson, F.; Hoste, H., 2010. *In vitro* methods for the primary screening of plant products for direct activity against ruminant gastrointestinal nematodes. Dans: Vercoe, P.E.; Makkar, H.P.S.; Schlink, A.C. (Eds.), *In vitro* screening of Plant Resources for Extra Nutritional Attributes in Ruminants: Nuclear and Related Methodologies, FAO/IAEA Springer Edition. pp 24-45.

Jackson, F., Varady, M., Bartley, D.J., 2012. Managing anthelmintic resistance in goats-Can we learn lessons from sheep? *Small Ruminant Res.*, 103, 3–9.

Jacquiet, P., 1997. Les strongles digestifs des ruminants. *Point Vét. (Parasitologie des ruminants),* 28, 20-22.

Jean-Blain, C., 1998. Aspects nutritionnels et toxicologiques des tanins. *Rev. Méd. Vét.,* 149, 911-920.

Kabasa, J.D., Opuda-Asibo, J., Ter Meulen, U., 2000. The effect of oral administration of polyethylene glycol on faecal helminth egg counts in pregnant goats grazed on browse containing condensed tannins. *Trop. Anim. Health Prod.,* 32, 73-86.

Kahiya, C., Mukaratirwa, S., Thamsborg, S.M., 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Vet. Parasitol.,* 115, 265–274.

Kahn, L.P., Diaz-Hernandez, A., 2000. Tannins with anthelmintic properties. In: Proc. International Workshop on Tannins in Livestock and Human Nutrition (Ed. J. D. Brooker), ACIAR Proceedings No.92. 171pp.

Kaminsky, R., Ducray, P., Jung, M., Clover, R., Rufener, L., Bouvier, J., Weber, S.S., Wenger, A., Wieland-Berghausen, S., Goebel, T., Gauvry, N., Pautrat, F., Skripsky, T., Froelich, O., Komoin-Oka, C., Westlund, B., Sluder, A., Maser, P., 2008. A new class of anthelmintics effective against drug- resistant nematodes. *Nature*, 452, 176–U119.

Kaminsky, R., Mosimann, D., Sager, H., Stein, P., Hosking, B., 2009. Determination of the effective dose rate for monepantel (AAD 1566) against adult gastro-intestinal nematodes in sheep. *Int. J. Parasitol.,* 39, 443–6.

Kaplan, R.M., 2004. Drug resistance in nematodes of veterinary importance: A status report. *Trends Parasitol.*, 20, 477–481.

Karanu, F.N., Mc Guire, T.C., Davis, W.C., Besser, T.E., Jasmer, D.P., 1997. CD4+ T lymphocites contribute to protective immunity induced in sheep and goats by *Haemonchus contortus* gut antigens. *Parasite Immunol*., 19, 435-445.

Kenyon, F., Jackson, F., 2012. Targeted flock/herd and individual ruminant treatment approaches. *Vet. Parasitol*., 186, 10–17.

Ketzis, J.K., Vercruysse, J., Stromberg, B.E., Larsen, M., Athanasiadou, S., Houdijk, J.G., 2006. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Vet. Parasitol.,* 139, 321-335.

Kidane, A., Houdijk, J., Athanasiadou, S., Tolkamp, B., Kyriazakis, I., 2010. Nutritional sensitivity of periparturient resistance to nematode parasites in two breeds of sheep with different nutrient demands. *Br. J. Nutr*., 104, 1477–1486.

Kidane, A., Houdijk, J.G.M., Tolkamp, B.J., Athanasiadou, S., Kyriazakis, I., 2009. Consequences of infection pressure and protein nutrition on periparturient resistance to *Teladorsagia circumcincta* and performance in ewes. *Vet. Parasitol*., 165, 78–87.

Knox, M.R., Torres-Acosta, J.F., Aguilar-Caballero, A.J., 2006. Exploiting the effect of dietary supplementation of small ruminants on resilience and resistance against gastrointestinal nematodes. *Vet. Parasitol.,* 139, 385-393.

Kotze, A.C., Hunt, P. W., Skuce, P., von Samson-Himmelstjerna, G., Martin, R.J., Sager, H., Krücken, J., Hodgkinson, J., Lespine, A., Jex, A.R., Gilleard, J.S., Beech, R.N., Wolstenholme, A.J., Demeler, J., Robertson, A.P., Charvet, C.L., Neveu, C.L., Kaminsky, R., Rufener, L., Alberich, M. Menez, C. et al., 2014. Recent advances in candidate-gene and whole-genome approaches to the discovery of anthelmintic resistance markers and the description of drug/receptor interactions. *Int. J. Parasitol: Drugs and Drugs Resistance*, 4, 164-184.

Koupai-Abyazani, M.R., Muir, A.D., Bohm, B.A., Towers, G.H.N., Gruber, M.Y., 1993. The proanthocynidin polymers in some species of Onobrychis. *Phytochemistry,* 34, 113-117.

Kozan, E., Anul, S.A., Tatli, I.I., 2013. *In vitro* anthelmintic effect of *Vicia pannoni*ca var. *purpurascens* on trichostrongylosis in sheep. *Exp. Parasitol*., 134, 299–303.

Kyriazakis, I., Anderson, D.H., Oldham, J.D., Coop, R.L., Jackson, F. 1996. Long-term subclinical infection with *Trichostrongylus colubriformis*: effects on food intake, diet selection and performance of growing lambs. *Vet. Parasitol*., 61, 297-313.

Kyriazakis, I., Houdijk, J., 2006. Immunonutrition: Nutritional control of parasites. *Small Ruminant Res.,* 62, 79–82.

Lacroux, C., 2006. Régulation des populations de Nématodes gastro-intestinaux (*Haemonchus contortus* et *Trichostrongylus colubriformis*) dans deux races ovines, INRA 401 et Barbados Black Belly. INP Toulouse, Toulouse.

Lange, K.C., Olcott, D.D., Miller, J.E., Mosjidis, J.A., Terrill, T.H., Burke, J.M., Kearney, M.T., 2006. Effect of sericea lespedeza (*Lespedeza cuneata*) fed as hay, on natural and experimental *Haemonchus contortus* infections in lambs. *Vet. Parasitol.,* 141, 273-278.

Lanusse, C.E., Prichard, R.K., 1993. Clinical pharmakocinetics and metabolism of benzimidazole anthelmintics in ruminants. *Drug Metab. Rev*., 25, 235-279.

Leathwick, D., Miller, C., McMurty, L., 2013. Resistance to monepantel in two nematode species in goats. World Association for the Advancement of Veterinary Parasitology: The 24th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP), Buenos Aires, Argentina, August 2013.

Leathwick, D.M., Besier, R.B. 2014. The management of anthelmintic resistance in grazing ruminants in Australasia—Strategies and experiences. *Vet. Parasitol.,* 204, 44-54.

Le Bourvellec, C., Bouchet, B., Renard, C.M.G.C., 2005. Non-covalent interaction between procyanidins and apple cell wall material. Part III: Study on model polysaccharides. *Biochim. Biophys. Acta - Gen.*, Subj. 1725, 10–18.

Legarto, J., Leclerc, M.C., 2007. Guide pour la conduite de pâturage caprin. Office d'Elevage., 212 pp.

Lespine, A., Ménez, C., Bourguinat, C., Prichard, R.K., 2012. P-glycoproteins and other multidrug resistance transporters in the pharmacology of anthelmintics: prospects for reversing transport-dependent anthelmintic resistance. *Int. J. Parasitol. Drugs Drug Resist.,* 2, 58-75.

Li, C., Leverence, R., Trombley, J.D., Xu, S., Yang, J., Tian, Y., Reed, J.D., Hagerman, A.E., 2010. High molecular weight persimmon (*Diospyros kaki* L.) proanthocyanidin: A highly galloylated, a-linked tannin with an unusual flavonol terminal unit, myricetin. *J. Agric. Food Chem.*, 58, 9033–9042.

López-Andrés, P., Luciano, G., Vasta, V., Gibson, T.M., Biondi, L., Priolo, A., Mueller-Harvey, I., 2013. Dietary quebracho tannins are not absorbed, but increase the antioxidant capacity of liver and plasma in sheep. *Br. J. Nutr.*, 110, 632–9.

Lorenz, M.M., Alkhafadji, L., Stringano, E., Nilsson, S., Mueller-Harvey, I., Udén, P., 2014. Relationship between condensed tannin structures and their ability to precipitate feed proteins in the rumen. *J. Sci. Food Agric.*, 94, 963–968.

Mahieu, M., 2013. Effects of stocking rates on gastrointestinal nematode infection levels in a goat/cattle rotational stocking system. *Vet. Parasitol.*, 198, 136–44.

Mallet, S., Lesage, M.C., 1987. Relationship between exsheathment and enzyme activity (alkaline phosphatase and leucine amino peptidase) during ageing of *Trichostrongylus culubriformis* infective larvae. *Ann. Rech. Vet.*, 18, 275-278.

Manolaraki, F., 2011. Propriétés anthelminthiques du sainfoin (*Onobrychis viciifolia*): Analyse des facteurs de variations et du rôle des composés phénoliques impliqués. Ph.D. Dissertation, INP, Université de Toulouse, Toulouse, France. 185 pp.

Manolaraki, F., Sotiraki, S., Stefanakis, A., Skampardonis, V., Volanis, M., Hoste, H. 2010. Anthelmintic activity of some Mediterranean browse plants against parasitic nematodes. *Parasitology*, 137, 685-696.

Mahl, D.L., Zanette, R.A., Jesus, F.P.K., Friedriczewski, A.B., Alves, S.H., Santurio, J.M., 2012. Animal-crop rotation system: A hurdle for the use of the nematophagous fungus *Duddingtonia flagrans*. *Biol. Control*, 62, 82–85.

Makkar, H.P.S., 2000. Quantification of tannins in tree foliage : Working document. In: *FAO/ IAEA*, Vienna.

Makkar, H.P., Dawra, R.K., Singh, B., 1991. Tannin levels in leaves of some oak species at different stages of maturity. *J. Sci. Food Agric.,* 54, 513- 519.

Makkar, H.P., Blummel, M., Becker, K., 1995. Formulation of complexes between polyvinyl pyrrolidones or polyethylene glycols and tannins, and their implication in gas production and true digestibility in *in vitro* techniques. *Br. J. Nutr.*, 73, 897-913.

Marais, J.P.J., Mueller-Harvey, I., Brandt, E.V., Ferreira, D., 2000. Polyphenols, condensed tannins and other natural products in *Onobrychis viciifolia* (sainfoin). *J. Agric. Food Chem.,* 48, 3440-3447.

Martínez-Ortíz-de-Montellano, C., Vargas-Magaña, J.J., Canul-Ku, H.L., Miranda-Soberanis, R., Capetillo-Leal, C., Sandoval-Castro, C. A., Hoste, H., Torres-Acosta, J.F.J., 2010. Effect of a tropical tannin-rich plant *Lysiloma latisiliquum* on adult populations of *Haemonchus contortus* in sheep. *Vet. Parasitol*., 172, 283–290.

Martínez-Ortíz-de-Montellano, C., Arroyo-López, C., Fourquaux, I., Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Hoste, H., 2013. Scanning electron microscopy of *Haemonchus contortus* exposed to tannin-rich plants under *in vivo* and *in vitro* conditions. *Exp. Parasitol.*, 133, 281–6.

Martínez-Valladares, M., Geurden, T., Bartram, D.J., Martínez-Pérez, J.M., Robles-Pérez, D., Bohórquez, A., Florez, E., Meana, A., Rojo-Vázqueza F.A., 2015. Resistance of gastrointestinal nematodes to the most commonly used anthelmintics in sheep, cattle and horses in Spain. *Vet. Parasitol.*, 211, 228–233.

Mc Kellar, Q.A. 1997. Ecotoxicity and residues of anthelminthic compounds. *Vet. Parasitol.,* 72, 413-435.

McAllister, T.A., Martinez, T., Hee, D.B., Muir, A.D., Yanke, L.J., Jones, G. A., 2005. Characterization of condensed tannins purified from legume forages: Chromophore production, protein precipitation, and inhibitory effects on cellulose digestion. *J. Chem. Ecol.*, 31, 2049–2068.

Min, B.R., Hart, S.P., 2002. Tannins for suppression of internal parasites. *J. Anim. Sci.,* 81, 102-109.

Min, B.R., Barry, T.N., Attwood, G.T., Mc Nabb, W.C., 2003. The effect of condensed tannins on the nutrition and health of ruminants fed fresh temperate forages: a review. *Anim. Feed Sci. Technol.* 106, 3-19.

Min, B.R., Pomroy, W.E., Hart, S.P., Sahlu, T., 2004. The effect of short-term consumption of a forage containing condensed tannins on gastro-intestinal nematode parasite infections in grazing whether goats. *Small Rum. Res*., 51, 279–283.

Min, B.R., Hart, S.P., Miller, D., Tomita, G.M., Loetz, E., Sahlu, T., 2005. The effect of grazing forage containing condensed tannins on gastro-intestinal parasite infection and milk composition in Angora does. *Vet. Parasitol.*, 130, 105–113.

Molan, A.L., Alexander, R.A., Brookes, I.M., Mcnabb, W.C., 2000. Effects of an extract from sulla (*Hedysarum coronarium*) containing condensed tannins on the migration of three sheep gastrointestinal nematodes *in vitro*. *Proc. New Zeal. Soc. Anim. Prod*., 60, 21–25.

Molan, A.L., Meagher, L.P., Spencer, P.A., Sivakumaran, S., 2003. Effect of flavan-3-ols on *in vitro* egg hatching, larval development and viability of infective larvae of *Trichostrongylus colubriformis. Int. J. Parasitol*., 33, 1691–1698.

Molan, A.L., Sivakumaran, S., Spencer, P. A., Meagher, L.P., 2004. Green tea flavan-3-ols and oligomeric proanthocyanidins inhibit the motility of infective larvae of *Teladorsagia circumcincta* and *Trichostrongylus colubriformis in vitro*. *Res. Vet. Sci.*, 77, 239–243.

Molento, M.B., Fortes, F.S., Pondelek, D.A.S., Borges, F.D.A., Chagas, A.C.D.S., Torres-Acosta, J.F.D.J., Geldhof, P., 2011. Challenges of nematode control in ruminants: Focus on Latin America. *Vet. Parasitol.,* 180, 126–132.

Moreno-Gonzalo, J., Manolaraki, F., Frutos, P., Hervás, G., Celaya, R., Osoro, K., Ortega-Mora, L.M., Hoste, H., Ferre, I., 2013a. *In vitro* effect of heather extracts on *Trichostrongylus colubriformis* eggs, larvae and adults. *Vet. Parasitol*., 197, 586–594.

Moreno-Gonzalo, J., Osoro, K., García, U., Frutos, P., Celaya, R., Ferreira, L.M.M., Ortega-Mora, L.M., Ferre, I., 2013b. Effect of the consumption of heather on incoming larvae and established population of *Teladorsagia circumcincta* in experimentally infected Cashmere goats. *Vet. Parasitol*., 196, 124–129.

Morris, C.A., Wheeler, M., Hosking, B.C., Wetson, T.G., Hurford, A.P., Foote, B.J., Foote, J.F., 1997. Genetic parameters for milk yield and feacal nematode egg count in Saanen does *N. Z. J. Agri. Res.,* 40, 523-528.

Mueller-Harvey, I., 2001. Analysis of hydrolysable tannins. *Anim. Feed Sci. Technol.,* 91, 3-20.

Mueller-Harvey, I., 2006. Unravelling the conundrum of tannins in animal nutrition and health. *J. Sci. Food Agric.*, 86, 2010–2037.

Mueller-Harvey, I., Mc Allan, A.B., 1992. Tannins: their biochemistry and nutritional properties. *Adv. Plant Cell Biochem. Biotechnol.,* 1, 151-217.

Mulligan, W., Gorrell, M.D., Rickard, M.D., Brandon, M.R., 1989. The use of irratiated larvae as immunising agents in *Haemonchus contortus* and *Trichostrongylus colubriformis* infections in sheep. *Aust. J. Agric. Res.,* 12, 1175-1187.

Nabukenya, I., Rubaire-Akiiki, C., Olila, D., Muhangi, D., Höglund J., 2014. Anthelmintic resistance in gastrointestinal nematodes in goats and evaluation of FAMACHA diagnostic marker in UgandA. *Vet. Parasitol*., 205, 666-675.

Naumann, H.D., Armstrong, S. A., Lambert, B.D., Muir, J.P., Tedeschi, L.O., Kothmann, M.M., 2014. Effect of molecular weight and concentration of legume condensed tannins on *in vitro* larval migration inhibition of *Haemonchus contortus*. *Vet. Parasitol*., 199, 93–98.

Niezen, J.H., Waghorn, T.S., Charleston, W.A., Waghorn, G.C., 1995. Growth and gastointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or sulla (*Hedysarum coronarium*) which contains condensed tannins. *J. Agric. Sci.,* 125, 281-289.

Niezen, J.H., Charleston, W.A.G., Hodgson, J., Mackay, A.D., Leathwick, D.M., 1996. Controlling internal parasites in grazing ruminants without recourse to anthelmintics : approaches, experiences and prospects. *Int. J. Parasitol.,* 26, 983-992.

Niezen, J.H., Robertson, H.A., Waghorn, G., Charleston, W.A., 1998a. Production, faecal egg counts and worm burdens of ewe lambs which grazed six contrasting forages. *Vet. Parasitol.,* 80, 15-27.

Niezen, J.H., Waghorn, G., Charleston, W.A., 1998b. Establishment and fecundity of *Ostertagia circumcincta* and *Trichostrongylus colubriformis* in lambs fed Lotus (*L.pedunculatus*) or perennial ryegrass (*Lolium perenne*). *Vet. Parasitol.,* 78, 13-21.

Novobilský, A., Mueller-Harvey, I., Thamsborg, S.M., 2011. Condensed tannins act against cattle nematodes. *Vet. Parasitol*., 182, 213–20.

Novobilský, A., Stringano, E., Hayot Carbonero, C., Smith, L.M.J., Enemark, H.L., Mueller-Harvey, I., Thamsborg, S.M., 2013. *In vitro* effects of extracts and purified tannins of sainfoin (*Onobrychis viciifolia*) against two cattle nematodes. *Vet. Parasitol*., 196, 532–7.

O'connor, L.J., Walkden-Brown, S.W., Kahn, L.P., 2006. Ecology of the free-living stages of major trichostrongylid parasites of sheep. *Vet. Parasitol.,* 142, 1-15.

Okuda, T., Ito, H., 2011. Tannins of constant structure in medicinal and food plants-hydrolyzable tannins and polyphenols related to tannins. *Molecules*, 16, 2191–2217.

Oliveira, L.M.B., Bevilaqua, C.M.L., Costa, C.T.C., Macedo, I.T.F., Barros, R.S., Rodrigues, A. C.M., Camurça-Vasconcelos, A. L.F., Morais, S.M., Lima, Y.C., Vieira, L.S., Navarro, A. M.C., 2009. Anthelmintic activity of *Cocos nucifera* L. against sheep gastrointestinal nematodes. *Vet. Parasitol.*, 159, 55–59.

Osoro, K., Celaya, R., Moreno-Gonzalo, J., Ferreira, L.M.M., García, U., Frutos, P., Ortega-Mora, L.M., Ferre, I., 2009. Effects of stocking rate and heather supplementation on gastrointestinal nematode infections and host performance in naturally-infected Cashmere goats. *Rangel. Ecol. Manag.*, 62, 127–135.

Page, A.P., 2001. The nematode cuticle: synthesis, modification and mutants. In: Kennedy MW, Harnett W, editors. Parasitic nematodes: molecular biology, biochemistry and immunology. London. CABI Publishing. pp. 167–93.

Paolini, V., Bergeaud, J.P., Grisez, C., Prevot, F., Dorchies, P., Hoste, H., 2003a. Effects of condensed tannins on goats experimentally infected with *Haemonchus contortus*. *Vet. Parasitol*., 113, 253–261.

Paolini, V., Frayssines, A., Farge, F., Dorchies, P., 2003b. Effects of condensed tannins on established populations and on incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia circumcincta* in goats. *Vet. Res*., 34, 331–339.

Paolini, V., Fouraste, I., 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., De La Farge, F., Prevot, F., Dorchies, P., Hoste, H., 2005a. Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. *Vet. Parasitol*., 127, 277–283.

Paolini, V., Prevot, F., Dorchies, P., Hoste, H., 2005b. Lack of effects of quebracho and sainfoin hay on incoming third-stage larvae of *Haemonchus contortus* in goats. *Vet. J.,* 170, 260–263.

Paraud, C., 2006. Contrôle biologique des nématodes parasites de la chèvre par le champignon nématophage *Duddingtonia flagrans.* Influence sur la biodiversité. Université de Poitier, Poitier.

Paraud, C., Lumaret, J.P., Chartier, C., 2007. Lack of effect of the nematophagous fungus Duddingtonia flagrans on the development of the dung beetle, Aphodius constans. *Small Rum. Res.*, 70, 276-279.

Patra, A.K., Saxena, J., 2011. Exploitation of dietary tannins to improve rumen metabolism and ruminant nutrition. *J. Sci. Food Agric*., 91, 24–37.

Pharmacopée-Européenne, 2001. Détermination des tanins dans les drogues végétales. 3ème Edition du conseil de l’Europe.

Pérez, J., Garcia, P., Hernandez, S., A., M.-M., Martin De Las Mulas, J., Camara, S., 2001. Pathological and immunohistochemical study of the abomasum and abomasal lymph nodes of goats experimentally infected with *Haemonchus contortus*. *J. Comp. Path.,* 138, 102-107.

Pérez, J., Garcia, P.M., Hernandez, S., Mozos, E., Camara, S., Martinez-Moreno, A., 2003. Experimental haemonchosis in goats: effects of single and multiple infections in the host response. *Vet. Parasitol.,* 111, 333-342.

Pérez, J., Zafra, R., Buffoni, L., Hernandez, S., Camara, S., Martinez-Moreno, A., 2008. Cellular phenotypes in the abomasal mucosa and abomasal lymph nodes of goats infected with *Haemonchus contortus*. *J. Comp. Pathol.,* 138, 102-107.

Pérez-Jiménez, J., Torres, J.L., 2011. Analysis of non-extractable polyphenols in foods: the current sate of the art. *J. Agric. Food Chem*., 59, 12713–12724.

Perez-Maldonado, R. A., Norton, B.W., 1996. The effects of condensed tannins from *Desmodium intortum* and *Calliandra calothyrsus* on protein and carbohydrate digestion in sheep and goats. *Br. J. Nutr*., 76, 515–533.

Perri, A.F., Mejía, M.E., Licoff, N., Lazaro, L., Miglierina, M., Ornstein, A., Becu-Villalobos, D., Lacau-Mengido, I.M., 2011. Gastrointestinal parasites presence during the peripartum decreases total milk production in grazing dairy Holstein cows. *Vet. Parasitol.*, 178, 311–318.

Piluzza G., Bullitta S., Deroma M., Odoardi M., 2000. The accumulation of condensed tannins in local populations of sulla. In : Sulas L. (ed.). Legumes for Mediterranean forage crops, pastures and alternative uses. Zaragoza : CIHEAM, 2000. p. 199-202. (Cahiers Options Méditerranéennes; n. 45). 10. Meeting of the Mediterranean Sub-Network of the FAO-CIHEAM Inter-Regional Cooperative Research and Development Network on Pastures and Fodder Crops, 2000/04/04-09, Sassari, Italy.

Pomroy, W.E., 2006. Anthelmintic resistance in New Zealand: a perspective on recent findings and options for the future. *N. Z. Vet. J.*, 54, 265-270.

Pomroy, W.E., Adlington, B.A., 2006. Efficacy of short-term feeding of sulla (*Hedysarum coronarium*) to young goats against a mixed burden of gastrointestinal nematodes. *Vet. Parasitol.*, 136, 363–366.

Poncet-Legrand, C., Edelmann, A., Putaux, J.L., Cartalede, D., Sarni-Machado, P., Vernhet, A., 2006. Poly(L-proline) interactions with flavan-3-ols units: influence of the molecular structure and the polyphenol/protein ratio. *Food Hydrocolloids*, 20, 687-697.

Porter, L.J., Hrstich, L.N., Chan, B.G., 1986. The conversion of procyanidins and prodelphinidins to cyanidins and delphinidins. *Phytochemistry*, 1, 223-230.

Rahman, W.A., Collins, G.H., 1990. The establishment and development of *Trichostrongylus colubriformis* in goats. *Vet. Parasitol.,* 35, 195-200.

Rahman, W.A., Collins, G.H., 1992. An association of faecal egg counts and prolactin concentrations in sera of periparturient Angora goats. *Vet. Parasitol.*, 43, 85–91.

Ramírez-Restrepo, C.A., Barry, T.N., López-Villalobos, N., Kemp, P.D., McNabb, W.C., 2004. Use of *Lotus corniculatus* containing condensed tannins to increase lamb and wool production under commercial dryland farming conditions without the use of anthelmintics*. Anim. Feed Sci. Technol.*, 117, 85–105.

Ramsay, A., Drake, C., Grosse Brinkhaus, A., Girard, M., Copani, G., Dohme-Meier, F., Bee, G., Niderkorn, V., Mueller-Harvey, I., 2015. Sodium hydroxide enhances extractability and analysis of proanthocyanidins in ensiled sainfoin (*Onobrychis viciifolia*). *J. Agric. Food Chem*., 63, 9471–9479.

Robinson, N., Pleasance, J., Piedrafita, D., Meeusen, E.N., 2011. The kinetics of local cytokine and galectin expression after challenge infection with the gastrointestinal nematode, *Haemonchus contortus*. *Int. J. Parasitol.*, 41, 487–93.

Rocha, R.A., Bresciani, K.D.S., Barros, T.F.M., Fernandes, L.H., Silva, M.B., Amarante, A.F.T., 2008. Sheep and cattle grazing alternately: Nematode parasitism and pasture decontamination. *Small Ruminant Res.,* 75, 135-143.

Rochfort, S., Parker, A.J., Dunshea, F.R., 2008. Plant bioactives for ruminant health and productivity. *Phytochemistry,* 69, 299-322.

Rogers, W.P. and Sommerville, R.I., 1963. The infective stage of nematode parasites and its significnace in parasitism. *Adv. Parasit.,* 1, 109-171.

Rogers, W.P. and Sommerville, R.I., 1968. The infectious process and its relation to the development of early parasitic stages of nematodes. *Adv. Parasit.* 6, 327-348.

Rolfe, P.F., Giraudel, J.M., MacKenzie, K., Browning, A., Allan, B., Sager, H., 2009. Efficacy, safety and pharmacokinetics of monepantel in goats. *N. Z. Soc. Parasitol. Proc. Annu. Meeting*, 37, 18.

Sager, H., Hosking, B., Bapst, B., Stein, P., Vanhoff, K., Kaminsky, R., 2009. Efficacy of the amino-acetonitrile derivative, monepantel, against experimental and natural adult stage gastro-intestinal nematode infections in sheep. *Vet. Parasitol*., 159, 49-54.

Salem, A.Z.M., López, S., Ranilla, M.J., González, J.S., 2013. Short- to medium-term effects of consumption of quebracho tannins on saliva production and composition in sheep and goats. *J. Anim. Sci.*, 91, 1341–1349.

Salminen, J.P., Karonen, M., 2011. Chemical ecology of tannins and other phenolics: We need a change in approach. *Funct. Ecol.*, 25, 325–338.

Salminen, J.P., Karonen, M., Sinkkonen, J., 2011. Chemical ecology of tannins: Recent developments in tannin chemistry reveal new structures and structure-activity patterns. *Chem. - A Eur. J.,* 17, 2806–2816.

Samsom-Himmelsjerna, G.V., 2007a. Mode of action of current anthelminthic drug classes. In: *Anthelminthics and resistance : a review*, NOVARTIS (Ed.), Switzerland.

Samsom-Himmelsjerna, G.V., 2007b. Mechanisms of resistance to anthelmintics in nematodes. In: *Anthelmintics and resistance*: *a review*, NOVARTIS (Ed.), Switzerland.

Sansger, N.C., Gill, J., 1999. Pharmacology of anthelmintic resistance. *Parasitol. Today,* 15, 141-146.

Santurio, J.M., Zanette, R.A., Da Silva, A.S., Fanfa, V.R., Farret, M.H., Ragagnin, L., Hecktheuer, P.A., Monteiro, S.G., 2011. A suitable model for the utilization of *Duddingtonia flagrans* fungus in small-flock-size sheep farms. *Exp. Parasitol*., 127, 727–31.

Schofield, P., Mbugua, D.M., Pell, A.N., 2001. Analysis of condensed tannins: a review. *Anim. Feed Sci. Technol.*, 9, 21-40.

Scott, I., Pomroy, W.E., Kenyon, P.R., Smith, G., Adlington, B., Moss, A., 2013. Lack of efficacy of monepantel against *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. *Vet. Parasitol*., 198, 166–71.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kaplan, R.M., Burke, J.M., Mosjidis, J.A., 2006. Sericea lespedeza hay as a natural deworming agent against gastrointestinal nematode infection in goats. *Vet. Parasitol.*, 139, 150–157.

Shakya, K.P., Miller, J.E., Lomax, L.G., Burnett, D.D., 2011. Evaluation of immune response to artificial infections of *Haemonchus contortus* in Gulf Coast Native compared with Suffolk lambs. *Vet. Parasitol*., 181, 239–47.

Silanikove, N., Perevolotsky, A., Provenza, F.D., 2001. Use of tannin-binding chemicals to assay for tannins and their negative postingestive effects in ruminants. *Anim. Feed Sci. Technol*., 91, 69–81.

Silva, A.R., Araújo, J. V, Braga, F.R., Alves, C.D.F., Frassy, L.N., 2010. *In vitro* ovicidal activity of the nematophagous fungi *Duddingtonia flagrans*, *Monacrosporium thaumasium* and *Pochonia chlamydosporia* on *Trichuris vulpis* eggs. *Vet. Parasitol*.,172, 76–9.

Simon, C., Barathieu, K., Laguerre, M., Schmitter, J.M., Fouquet, E., Pianet, I., Dufourc, E.J., 2003. Three-dimensional structure and dynamics of wine tannin-saliva protein complexes. A multitechnique approach. *Biochemistry,* 42, 10385-10395.

Smith, W.D., Sherman, D.M. 2009. Goat Medicine. Wiley-Blackwell, 2nd edition, Baltimore, USA. 888 pp.

Smith, W.D., van Wyk, J.A., van Strijp, M.F., 2001. Preliminary observations on the potential for gut membrane proteins of *Haemonchus contortus* as candidate vaccine antigens in sheep on naturally infected pasture. *Vet. Parasitol.*, 98, 285–297.

Stojadinovic, M., Radosavljevic, J., Ognjenovic, J., Vesic, J., Prodic, I., Stanic-Vucinic, D., Cirkovic Velickovic, T., 2013. Binding affinity between dietary polyphenols and Β-lactoglobulin negatively correlates with the protein susceptibility to digestion and total antioxidant activity of complexes formed. *Food Chem*. 136, 1263–1271.

Sumano, H., Ocampo, L., 1997. Farmacología Veterinaria. 2ª Ed. McGraw-Hill Interamericana. Mexico. 680 pp.

Sutherland, I. A., Leathwick, D.M., 2011. Anthelmintic resistance in nematode parasites of cattle: A global issue? *Trends Parasitol.*, 27, 176–181.

Sykes, A.R., Coop, R.L., 1976. Intake and utilization of food by growing lambs with parasitic damage to the small intestine caused by daily dosing with *Trichostrongylus colubriformis* larvae. *J. Agric. Sci.,* 86, 507-515.

Sykes, A.R., Coop, R.L., 1977. Intake and utilization of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. *J. Agric. Sci.,* 88, 671-677.

Tariq, K.A., Chishti, M.Z., Ahmad, F., Shawl, A.S., 2008. Epidemiology of gastrointestinal nematodes of sheep managed under traditional husbandry system in Kashmir valley. *Vet. Parasitol*., 158, 138–43.

Terefe, G., Lacroux, C., Andreoletti, O., Grisez, C., Prevot, F., Bergeaud, J.P., Penicaud, J., Rouillon, V., Gruner, L., Brunel, J.C., Francois, D., Bouix, J., Dorchies, P., Jacquiet, P., 2007. Immune response to *Haemonchus contortus* infection in susceptible (INRA 401) and resistant (Barbados Black Belly) breeds of lambs. *Parasite Immunol*., 29, 415–424.

Terrill, T.H., Douglas, G.B., Foote, A.G., Purchas, R.W., Wilson, G.F., Barry, T.N., 1992. Effects of condensed tannins upon body growth, wool growth and rumen metabolism in sheep grazing sulla (*Hedysarum coronarium*) and perennial pasture. *J. Agric. Sci.,* 119, 265-273.

Terrill, T.H., Waghorn, G.C., Woolley, D.J., McNabb, W.C., Barry, T.N., 1994. Assay and digestion of ^{14}C-labelled condensed tannins in the gastrointestinal tract of sheep. *Br. J. Nutr*., 72, 467–477.

Terrill, T.H., Mosjidis, J.A., Moore, D.A., Shaik, S.A., Miller, J.E., Burke, J.M., Muir, J.P., Wolfe, R., 2007. Effect of pelleting on efficacy of sericea lespedeza hay as a natural dewormer in goats. *Vet. Parasitol.*, 146, 117-122.

Terrill, T.H., Dykes, G.S., Shaik, S.A., Miller, J.E., Kouakou, B., Kannan, G., Burke, J.M., Mosjidis, J. A., 2009. Efficacy of sericea lespedeza hay as a natural dewormer in goats: dose titration study. *Vet. Parasitol.*, 163, 52–6.

Thamsborg, S.M., Mejer, H., Bandier, M., Larsen, M., 2003. Influence of different forages on gastrointestinal nematode infections in grazing lambs. In: The 19th International Conference of WAAVP, New Orleans, USA. p. 189.

Tibe, O., Meagher, L.P., Fraser, K., Harding, D.R.K., 2011. Condensed tannins and flavonoids from the forage legume sulla (*Hedysarum coronarium*). *J. Agric. Food Chem.,* 59, 9402-9409.

Torres-Acosta, J.F., 1999. Supplementary feeding and the control of gastrointestinal nematodes of goats in Yucatan. The Royal Veterinary College. University of London, London.

Torres-Acosta, J.F.J., Hoste, H., 2008. Alternative or improved methods to limit gastro-intestinal parasitism in grazing sheep and goats. *Small Ruminant Res*., 77, 159–173.

Torres-Acosta, J.F.J., Sandoval-Castro, C. A., Hoste, H., Aguilar-Caballero, A. J., Cámara-Sarmiento, R., Alonso-Díaz, M. A., 2012. Nutritional manipulation of sheep and goats for the control of gastrointestinal nematodes under hot humid and subhumid tropical conditions. *Small Ruminant Res.*, 103, 28–40.

Tzamaloukas, O., Athanasiadou, S., Kyriazakis, I., Huntley, J.F., Jackson, F., 2006. The effect of chicory (*Cichorium intybus*) and sulla (*Hedysarum coronarium*) on larval development and mucosal cell responses of growing lambs challenged with *Teladorsagia circumcincta. Parasitology,* 132, 419-426.

Tzamaloukas, O., Athanasiadou, S., Kyriazakis, I., Jackson, F., Coop, R.L., 2005. The consequences of short-term grazing of bioactive forages on established adult and incoming larvae populations of *Teladorsagia circumcincta* in lambs. *Int. J. Parasitol.*, 35, 329–35.

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1996. Veterinary Parasitology, 2nd ed., Oxford. 307 pp.

Vagenas, D., Jackson, F., Russel, A.J.F., Merchant, M., Wright, I.A., Bishop, S.C., 2002. Genetic control of resistance to gastro-intestinal parasites in crossbred cashmere-producing goats: responses to selection, genetic parameters and relationships with production traits. *Anim. Sci.,* 74, 199-208.

Van den Brom, R., Moll, L., Kappert, C., Vellema, P., 2015. Monepantel resistance of *Haemonchus contortus* in a Dutch sheep flock urges the application of preventive management measurements. Proceedings: 25th International Conference of the World Association for the Advancement of Veterinary Parasitology (WAAVP). 16-20 August, 2015. Liverlpool, UK, p. 238.

Van Houtert, M.F.J., Sykes, A.R., 1996. Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. *Int. J. Parasitol.*, 26, 1151–1167.

Van Wyk, J.A., 2001. Refugia – overlooked as perhaps the most important factor concerning the development of anthelmintic resistance. *Onderstepoort J. Vet. Res.*, 68, 55–67.

Van Wyk, J.A., Bath, G.F., 2002. The FAMACHA system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. *Vet. Res.,* 33, 509-529.

Van Wyk, J.A., Hoste, H., Kaplan, R.M., Besier, R.B., 2006. Targeted selective treatment for worm management-How do we sell rational programs to farmers? *Vet. Parasitol.*, 139, 336–46.

Vargas-Magaña, J.J., Aguilar-Caballero, A. J., Torres-Acosta, J.F.J., Sandoval-Castro, C. a, Hoste, H., Capetillo-Leal, C.M., 2013. Tropical tannin-rich fodder intake modifies saliva-binding capacity in growing sheep. *Animal*, 7, 1921–1924.

Veneziano, V., Rinaldi, L., Caputo, A.R., Fedele, V., Gringoli, G., 2007. Effects of gastrointestinal strongyle parasitism on milk quality. In: *The quality of goat products* (IGA-CRA, ed.), pp. 142-145, Bella, Italy.

Vercruysse, J., 1983. A survey of seasonal changes in nematode faecal egg count levels of sheep and goats in Senegal. *Vet. Parasitol.,* 13, 239-244.

Waghorn, G. 2008. Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production-Progress and challenges. *Anim. Feed Sci. Technol.,* 147, 116-139.

Waghorn, T.S., Molan, A.L., Deighton, M., Alexander, R.A., Leathwick, D.M., Mc Nabb, W.C., Meagher, L.P., 2006. *In vivo* anthelmintic activity of *Dorycnium rectum* and grape seed extract against *Ostertagia (Teladorsagia) circumcincta* and *Trichostrongylus colubriformis* in sheep. *N. Z. Vet. J.* 54, 21-27.

Waller, P.J., 2004. Management and control of nematode parasites of small ruminants in the face of total anthelmintic failure. *Trop. Biomed.*, 21, 7-13.

Waller, P.J., 2006a. From discovery to development: current industry perspectives for the development of novel methods of helminth control in livestock. *Vet. Parasitol.,* 139, 1-14.

Waller, P.J., 2006b. Sustainable nematode parasite control strategies for ruminant livestock by grazing management and biological control. *Anim. Feed Sci. Technol*., 126, 277–289.

Waller, P.J., Thamsborg, S.M., 2004. Nematode control in 'green' ruminant production systems. *Trends Parasitol.,* 20, 493-497.

Wang, Y., McAllister, T., Acharya, S., 2015. Condensed tannins in sainfoin: composition, concentration, and effects on nutritive and feeding value of sainfoin forage. *Crop Sci*., 55, 13-22.

Wang, T., Van Wyk, J. A., Morrison, A., Morgan, E.R., 2014. Moisture requirements for the migration of *Haemonchus contortus* third stage larvae out of faeces. *Vet. Parasitol.*, 204, 258–264.

Waterman, P.G., 1999. The tannins - An overview. In: Tannins in Livestock and Human Nutrition. Proceedings of an International Workshop, BROOKER (Ed.), Adelaide, Australia, pp. 10-13.

Weisshaar, B., Jenkins, G.J., 1998. Phenylpropanoid biosynthesis and its regulation. *Curr. Opin. Plant Biol.,* 1, 251-257.

Werne, S., Perler, E., Maurer, V., Probst, J.K., Hoste, H., Drewek, A., Heckendorn, F., 2013. Effect of sainfoin (*Onobrychis viciifolia*) and faba bean (*Vicia faba*) on the periparturient rise in ewes infected with gastrointestinal nematodes. *Small Ruminant Res.*, 113, 454–460.

Williams, A.R., Karlsson, L.J.E., Palmer, D.G., Vercoe, P.E., Williams, I.H., Greeff, J.C., Emery, D.L., 2010. Relationships between faecal dry matter, worm burdens and inflammatory mediators and cells in parasite-resistant Merino rams. *Vet. Parasitol.*, 171, 263–72.

Williams, A.R., Douglas, A.D., Miura, K., Illingworth, J.J., Choudhary, P., Murungi, L.M., Furze, J.M., Diouf, A., Miotto, O., Crosnier, C., Wright, G.J., Kwiatkowski, D.P., Fairhurst, R.M., Long, C.A., Draper, S.J., 2012. Enhancing blockade of Plasmodium falciparum erythrocyte invasion: assessing combinations of antibodies against PfRH5 and other merozoite antigens. *PLoS Pathog.*, 8 (11), e1002991.

Williams, A.R., Fryganas, C., Ramsay, A., Mueller-Harvey, I., Thamsborg, S.M., 2014. Direct anthelmintic effects of condensed tannins from diverse plant sources against *Ascaris suum*. *PLoS One 9*. DOI: 10.1371/journal.pone.0097053.

Williamson, E.M., 2001. Synergy and other interactions in phytomedicines. *Phytomedicine* 8, 401-409.

Williamson, A.L., Brindley, P.J., Knox, D.P., Hotez, P.J., Loukas, A., 2003. Digestive proteases of blood-feeding nematodes. *Trends Parasitol.*, 19, 417-423.

Windon, R.G. 1996. Genetic control of resistance to helminths in sheep. *Vet. Immunol. Immunopathol.,* 54, 245-254.

Wolstelholme, A.J., Fairweather, I., Prichard, R.K., Samson-Himmelstjerna, G., Sangster, N.C., 2004. Drug resistance in veterinary helminths. *Trends parasitol.*, 20, 469-476.

Wood, I.B., Amara, N.K. l, Bairden, K., Duncan, J.L., Kassai, T., Malone Jr., J.B., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., Vercruysse, J., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Vet. Parasitol.*, 58, 181-213.

Woodward, A., Coppock, D.L., 1995. Role of plant defense in the utilization of native browse in southern Ethiopia. *Agrofores. Syst.,* 32, 147-161.

Yue, C., Coles, G.C., Blake, N., 2003. Multiresistant nematodes on a Devon farm. *Vet. Rec.,* 153, 604.

jjqp