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The anthelmintic properties of tannin-rich legume forages: from knowledge to exploitation in farm conditions

H. Hoste*[1], F. Manolaraki*, S. Brunet*, C. Arroyo López*, C. Martínez-Ortiz de Montellano*,*, S. Sotiraki**, F. Torres Acosta*****

*UMR 1225 INRA/DGER. Ecole Nationale Vétérinaire de Toulouse
23 Chemin des Capelles, 31076 Toulouse Cedex (France)
**NAGREF-VRI NAGREF Campus, Thermi 57001 PO Box 60272 Thessaloniki (Greece)
***Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Yucatán,
Km 15.5 Carretera Mérida-Xmatkuil. Mérida, Yucatán (México)
[1]e-mail: h.hoste@envt.fr

Abstract. Tannin-rich legume forages have nutraceutical value for ruminants through slowing down the infections with parasitic nematodes of the gastro intestinal tract. This review aims to describe the effects of tannin-containing legumes on the various nematode stages We will also discuss the sources of variability in results and present the current hypotheses in regard to the possible modes of action of the plant secondary metabolite on the worms.

Keywords. Legume forage – Plant secondary metabolites – Tannin – Nematode – Ruminant – Nutraceutical.

Propriétés antihelminthiques des plantes fourragères légumineuses riches en tanins : de la connaissance vers l'utilisation pratique dans des exploitations

Résumé. *Les plantes fourragères légumineuses riches en tanins ont une valeur nutraceutique pour les ruminants étant donné qu'elles freinent les infections par nématodes parasites gastro-intestinaux. Cet article de révision décrit l'effet des légumineuses qui contiennent des tanins aux différents stades des nématodes. Il y aura également une discussion sur les sources de variabilité des résultats et les hypothèses actuelles sur les modes d'action possibles qu'ont les métabolites secondaires des plantes sur les vers.*

Mots-clés. *Légumineuses fourragères – Métabolites secondaires des plantes – Tanins – Nématodes – Ruminants – Nutraceutique.*

I – Background

Parasitic Nematodes of the gastro intestinal (GI) tract remain a major, worldwide issue for the health and the welfare of grazing ruminants, because of the pathological consequences and the related production losses that they provoke. Over the past decades, the control of these parasitic diseases has usually relied on the repeated, strategic (preventive) or tactic (curative) use of chemical anthelmintics (AHs). However, this nearly exclusive reliance on synthetic molecules is nowadays facing several limits. One is the enhanced concern of consumers on the use of chemicals in farm animals, generating possible residues in food products or having environmental consequences (Mc Kellar, 1997). This led to a trend for increased restrictions in the use of chemical drugs, either as medicinal products or food additives, through regulations at national or supranational (EU) levels. This also explains partly the current trend for products issued from organic farming systems in developed countries. In developing countries, the access of small farmers to chemical AHs is also usually limited for financial or practical reasons (Krecek and Waller, 2006).

However, the main threat to the use of chemical drugs for the control of GIN comes from the worms themselves. The rapid development of resistance to AHs in worm populations after commercialisation (Waller, 2006) and the worldwide diffusion of AH resistance within worm populations (Jackson and Coop, 2000; Kaplan, 2004) are the dominant reasons for the strong impetus given to current researches on alternative solutions (Waller, 2003; Krecek and Waller, 2006). Among those, the possible use of bioactive plants, rich in secondary metabolites, has been identified as a valuable solution to modulate the biology of parasitic nematodes and consequently to counteract the negative effects in the hosts. Some recent results indeed indicate that bioactive plants might represent a promising option to reduce nematode infections in small ruminants (see reviews by Githiori *et al.*, 2006, Hoste *et al.*, 2006).

II – Natural products as alternative solutions

The use of plants to treat humans and animals has been recorded since the early antiquity. At the start of the 20th century, plants or plant extracts represented worldwide, the core of remedies, both in human and veterinary medicines. It is noteworthy that, in many parts of the world, plants still remain for farmers, the basis of the Pharmacopea through ethnoveterinary sources. Also, it has been estimated that more than 30% of the recently introduced drugs are directly or indirectly issued from natural origins (Wilcox *et al.*, 2001). The traditional use of plants has mainly relied on the preparation of extracts applied as herbal remedies, although a novel option to exploit plants as nutraceuticals is nowadays evaluated (Min *et al.*, 2003, Waller and Thamsborg, 2004, Hoste *et al.*, 2006).

• *Phytotherapeutic drugs* are preparations of plants and/or of plant extracts whose administration, for a restricted, short term period, aims at treating infected animals. Curative effects are expected. In many cases, these herbal remedies are composed of mixed plants or plant extracts obtained through various physical and/or chemical processes. Aphytotherapeutic drug usually includes more than 50 biochemical molecules or components, whose geographical origins and relative proportions are not always well specified. This complexity is a characteristic of plant drugs and has consequences in the definition of the products and the validation of efficacy.

• *Nutraceuticals*. The term *nutraceutical* is issued from a contraction between *nutrition* and *pharmaceutical*. It is defined as "any substance that may be considered a food or part of a food which provides health benefits, including the prevention and treatment of disease" (Andlauer and Furst, 2002). Nutraceuticals remain used as a feed resource but the first reason of their exploitation relates to their potential, beneficial effects on animal health. In contrast to phytotherapeutic drugs, the first aim of nutraceuticals is to prevent disease. This is usually achieved by a relatively long term distribution (at least for several days). In the case of infection of the GI tract with parasitic nematodes, the concept of nutraceuticals has first been illustrated by studies on forages which were previously known for their nutritive values (Niezen *et al.*, 1996; 1998; Waller and Thamsborg, 2004). Like for the herbal remedies, the presence of plant secondary metabolites (PSMs) is usually evoked to explain the beneficial effects on animal health. For example, sesquiterpene lactones are suspected to be the biochemical compounds explaining the AH effects of chicory (Hoste *et al.*, 2006, Foster *et al.*, 2009). However, most of the examples of nutraceuticals with activity against nematodes have been obtained with tannin-rich (TR) plants.

Some studies have explored the potential value as nutraceuticals represented by several TR browses exploited in temperate, Mediterranean (Hoste *et al.*, 2009, Osoro *et al.* 2007) or under a large range of tropical conditions, (e.g. *Leucaena leucocephala* (Alonso Diaz *et al.*, 2008a, 2008b; Ademola *et al.*, 2006), various species of *Acacia spp* (Cenci *et al.*, 2007, Kahiya *et al.*, 2003, Akkari *et al.*, 2008), cassava (*Manihot esculenta*) (Sokerya, 2009), or a range of Mexican browse species (Alonso Diaz *et al.*, 2008a and b). However, during the last 15 years, the bulk of results on nutraceuticals in parasitized ruminants and the possible applications in farm conditions have been related to the consumption by tannin-rich (TR) legume forages. This short review will focus on these Legumes in order to illustrate what are the effects on the parasite biology, which are the possible mechanisms of action and how nutraceuticals can be exploited in farm condition.

III – TR forages and AH properties

The first step to identify the AH properties of TR forages, has relied on some *in vitro* assays targeting various key stages of the GI Nematode cycle: i.e. the egg (Egg Hatch Assay = EHA), the development of egg to larvae (Larval Development Assay : LDA), the first and second stage larvae (Larval Feeding Inhibition Assay = LFIA), third stage larvae (Larval Migration Inhibition assay = LMIA) and Larval Exsheathment Inhibition Assay = =LEIA) and Adult worms (Adult Motility Inhibition Assay = AMIA). This range of *in vitro* tests enables to screen the potential AH activity of plant extracts. With some adaptations, most of these assays are derived from the usual ones developed to assess in vitro the efficacy of chemical drugs (Wood *et al.*, 1995).

The list of TR fodders from the Fabaceae family which have been screened for potential AH activity based on such in vitro assays is relatively long. However, up to now, consistent results, based on the confirmation in *in vivo* studies of early *in vitro* results, concerned mainly five temperate tannin-rich (TR) Legume forages, i.e. sulla (*Hedysarum coronarium) (*Niezen *et al.*, 1995, 1998a, 2002a); big trefoil (*Lotus pedunculatus)* and birdfoot trefoil (*Lotus corniculatus*) (Niezen *et al.*, 1995, 1998b, Marley *et al.*, 2003, Heckendorn *et al.*, 2007*),* Chinese bush clover = sericea lespedeza (*Lespedeza cuneata*) (Shaik *et al.*, 2006, Lange *et al.*, 2006; Terrril *et al.*, 2007 and 2009, Min *et al.*, 2004, 2005) or sainfoin (*Onobrychis viciifolia*) (Paolini *et al.*, 2003c, 2005, Heckendorn *et al.*, 2006, 2007). These five plants will provide the core of results for this short review.

From the *in vivo* results, the consumption of TR forages by sheep or goats has been associated with a modulation of the nematode biology explained by impacts on different key stages of the life cycle (Fig. 1).

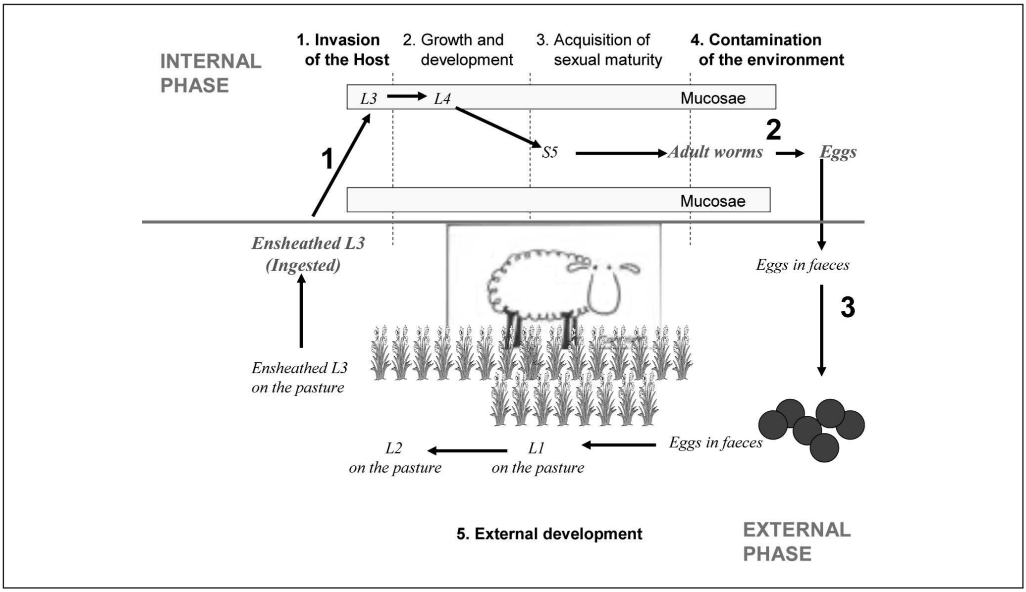

Fig. 1. The impact of the consumption of TR forages has been reported on 3 key stages of the GI nematode life cycle: 1/ the infective, third-stage larvae (L3); 2/ the adult worm and 3/ the egg development.

1. A decreased establishment of the infective third-stage larvae which contributes to reduce the invasion of the host (Paolini et al, 2003b, Lange *et al.*, 2006).

2. A decreased excretion of nematode eggs which contributes to reduce the pasture contamination. This widely described process has been related either to a reduced number of worms (Heckendorn *et al.*, 2006, 2007, Lange *et al.*, 2006, Shaik *et al.*, 2006, Terril *et al.* 2007) or to a reduced fertility of female worms (Paolini *et al.*, 2003a and b, 2005).

3. A decreased development of eggs to infective larvae in the faeces and /or on the pastures which contributes to reduce the pasture infectivity for the sheep or the goats (Niezen *et al.*, 2002b, Marley *et al.*, 2003b, Min *et al.*, 2004, Shaik *et al.*, 2006). However, the results concerning this last impact were fewer and less consistent than for the two first aspects.

In addition, the consumption of TR forages has usually been associated with a better host resilience, as mentioned for sulla (Niezen *et al.*, 1995, 1998a), sericea lespedeza (Min *et al.*, 2005, Shaik *et al.*, 2006), sainfoin (Paolini *et al*. 2005) and to a lesser extent with *Lotus pedunculatus* (Niezen *et al.*, 1998b).

By comparison to chemical AHs, one the main characteristics of these nutraceuticals is that the main effect seems more a regulation of the worm biology than worm lethality. The second main difference is the occurrence of variability in results reported in the various studies. Several factors have been described as possible origins of this variability.

IV – Variability of effects on GI nematodes with tannin-rich fodders

1. Parasite species

The results of several *in vivo* studies, in sheep or goats, receiving the same TR Legume forages suggest that the AH effects of bioactive Legumes depend on the nematode species. For example, the consumption of sainfoin by goats has been associated with reductions of larval establishment of respectively 70, 66 and 33% for *Teladorsagia circumcincta, Trichostrongylus colubriformis* or *Hameonchus contortus (*Paolini *et al.*, 2003 a and 2003b*)*. Some authors have suggested that the anatomical location of the Nematode might represent a major factor to consider since divergent effects were found between the abomasal (*H. contortus / T. circumcincta*) vs the intestinal species (*Trichostrongylus colubriformis, Nematodirus* sp.*, or Cooperia* sp.). For example, by giving quebracho, a tannin purified resource to sheep, Athanasiadou *et al.* (2001) found significant effects against the intestinal species but not against the abomasal ones. In contrast, with sainfoin, Heckendorn *et al.* (2006 and 2007) found more severe consequences on the number of abomasal *Haemonchus* than on the intestinal *Cooperia*. With sericea lespedeza hay, significant reductions of -70%, -26% and -40% were reported respectively for *H. contortus, T. circumcincta* and *T.colubriformis* in naturally infected goats (Shaik *et al.*, 2006). Similar values of reductions were found for *Haemonchus* and *Trichostrongylus* with pellets of sericea hay (Terrill *et al.*, 2007).

Overall, the comparison of *in vitro* result obtained on different parasite species suggest that the sensitivity to tannins or to extracts of tannin-rich forages is not species specific. The results obtained by the same assay were found similar between intestinal vs abomasal parasite species with quebracho (Athanasiadou *et al.*, 2001) or sainfoin (Paolini *et al.*, 2004). In contrast, with sulla extracts, the inhibition of migration of third stage of abomasal species was significantly greater than for *T. colubriformis* (Molan *et al.*, 2000).

Alternatively, it has been assumed that the variability in the *in vivo* effects might be first related to difference in the interactions between tannins and the worms depending on the environmental and/or physiological conditions. *In vivo* experiments suggest that the general binding of tannins to proteins is a pH dependent phenomenon (Mueller Harvey 2006). Therefore, the local conditions can widely influence the pharmacology of tannins by modifying the availability of free tannins and their ability to interact with Nematodes.

2. Host species

By reference to the feeding behaviour, beef and sheep are classically described as “grazers” whereas deer and goats are respectively classified as “browsers” or “intermediate browsers” (Gordon, 2003). This classification also refers to the ability of each species to adapt to the ingestion of PSMs, including tannins, and to counteract the possible negative consequences of these biochemical compounds. However, the presence of such adaptive mechanisms might also explain variations in the efficiency of TR forage between sheep *vs* goat and deer.

Because of the differences in experimental designs between studies, particularly in the fodders used, only a limited number of studies allow the comparison between sheep and goats. The results obtained with quebracho, a tannin-containing purified resource, or with sainfoin hay, fed to the animals, tend to indicate a reduction of some worm species in sheep (Athanasiadou *et al.*, 2001, Heckendorn *et al.*, 2006) whereas the main effect in goats seem to affect the worm fertility and the production of eggs (Paolini *et al.*, 2003a and 2003b, Paolini *et al.*, 2005).

3. Plants

Some of the variations in results on helminths observed between studies have been attributed to the plant resources consumed. Both qualitative and quantitative factors, affecting the PSMs and particularly the tannins, have been proposed to explain this variability.

Condensed tannins (CT) (= proanthocyanidins) and hydrolyzable tannins (HT) are the two major classes of tannins. Proanthocyanidins are flavonoid polymers. Hydrolyzable tannins are polymers of gallic or ellagic acid esterified to a core molecule, commonly glucose or a polyphenol such as catechin. Proanthocyanidins are the most common type of tannin found in forage legumes. (Reed, 1995). Based on their biochemical structures and the nature of the constitutive monomer (flavan-3-ol), four classes are distinguished within the CTs: the prodelphinidins, procyanidins, prorobetinidins and profisetinidins. In legume forages, prodelphinidins (PDs) and procyanidins (PCs) are mainly found, but the ratio between PDs and PCs differs between plant species and/or varieties (Mueller Harvey *et al.*, 2006).

By measuring experimentally the effects of different flavan-3-ols (monomers) constitutive of either prodelphinidins or procyanidins, some results suggest that PDs have more potent AH activity than PCs (Molan *et al*., 2003; Brunet *et al.*, 2006, 2008). This hypothesis is also supported by the fact that Legume fodders with high values of PD/PC (e.g. sainfoin, sericea lespedeza or *Lotus pedunculatus*) have shown more consistently AH activity than the species with a low PD/PC ratio (*Lotus corniculatus*) (Molan *et al.*, 2003).

The variation in AH property found between plants has also been related to the content of plant secondary metabolites (PSM). A dose dependent relationships between the AH efficacy and the concentration of plant extracts and/or PSMs (tannins or flavonoids) has been widely confirmed in *in vitro* assays (Barrau *et al.*, 2005, Molan *et al.*, 2003, Paolini *et al.*, 2004). A meta analysis performed by Min and Hart (2003) based on a literature review referring to data acquired with both legumes and quebracho, illustrate the occurrence of such a dose relationship *in vivo*. This relationship was also confirmed in experimental studies, performed either in sheep receiving an increased gradient of quebracho (Athanasiadou *et al.*, 2001) or in goats receiving different levels of sericea lespedeza (Terrill *et al.*, 2009). Overall, The results suggest that 4 to 5% of extractable condensed tannins are the requested threshold in order to obtain some AH effects, although as previously mentioned, the structure of tannins might play also a role.

It is known that both genetic and environmental factors influence the content and composition of secondary metabolites in plants, including Legume forages, like sainfoin (Koupai Abyazani *et al*., 1993a,b; Marais *et al.*, 2000). By using sainfoin as a model, a recent study aims at examining the role of these various factors in the AH activity of extracts measured by the LMIA. Overall, the results confirmed the dose-effect relationship. They have also shown that both the geographical origin and the phenological stages of *Onobrychis viciifolia* were two main factors influencing the AH efficacy, in relation with variations in tannin contents.

It is also suspected that technological treatments (drying or fermenting processes) aiming at a better conservation of Legume fodders can decrease the PSM contents and/or activity, including tannins (Makkar *et al.*, 2003). The early results on the AH properties of sulla, *Lotus corniculatus* and *L. pedunculatus* were acquired on fresh, grazed plants (Niezen *et al.*, 1995,1998a and 1998b) However, because of the practical advantages, for application in farm conditions and for possible measurement of tannin content before use, the AH activity of some conserved forms has rapidly been explored. For hay, the first positive data were obtained with sainfoin (Paolini *et al.*, 2003) and were thereafter completed by studies with the same plant (Paolini *et al.*,2005; Heckendorn *et al.* 2007) or on sericea lespedeza (Shaik *et al.*, 2006; Lange *et al.*, 2006). More recently, Heckendorn *et al.* (2006) examined whether the fermenting process involved in ensilaging also modify the AH properties of sainfoin. They found similar AH properties between hay and silage. This preservation of AH activity after a ensiling process has also been reported with cassava (*Manihot esculenta*) silage *vs* fresh plant by Sokerya (2009). Last in a recent study, Terrill *et al.* (2007) have shown that the pelleting of sericea lespedeza hay also preserved the AH properties, while facilitating the distribution to ruminants.

These results illustrate the progresses obtained on our understanding of the factors which modulate the AH activity of TR legume forages on parasitic Nematodes. Obviously, some questions related to the mechanisms of action have begun to be addressed: Which is/are the active compound(s)? How much is necessary? However, more basic studies (e.g. by exploring the relationship between the tannin structure and antiparasitic activity) are clearly needed to better identify the origin of the variability in the AH properties of legume forage. The better understanding of the interactions between TR legumes and the nematodes supposes also to examine another question which is : how do tannins and other PSMs affect the worm ? Up to now, only a few studies have been dedicated to this approach.

From the *in vivo* results, the consumption of TR forages by sheep or goats has been associated with a modulation of the nematode biology explained by impacts on different key stages of the life cycle (Figure 1).

V – Direct *vs* indirect mechanisms of action on nematodes

Two main, non exclusive, hypotheses (“direct” *vs* “indirect”) have been evoked to explain the effect of TR legume forages against parasitic GI nematodes in ruminants.

(i) Tannins might *directly* affect, by a pharmacological process, the biology of the worms and consequently modulate the epidemiology of GIN infections.

(ii) Alternatively, tannins may act *indirectly*, through the improvement of the host response against the worms. Because of their binding ability, tannins protect proteins of the ruminal degradations. Consequently, they favour an increased intestinal protein/peptide flow and amino acid absorption. It is known that any increase in the metabolizable proteins favours the two components of the host response (resistance and resilience) to nematodes (Coop and Kyriazakis, 2001). This so called “indirect hypothesis” has thus been put forward to explain the AH efficacy of TR forages.

The direct hypothesis has been supported by repeated and consistent results acquired through *in vitro* assays and, to a lower extent, in short-term experimental *in vivo* studies. In contrast, only a few studies have addressed the indirect hypothesis, by comparing the local cellular changes related to host immunity in the digestive mucosae of animals receiving or not TR forages. Overall, the results of the few studies performed in either sheep or goats did not shown main and consistent changes in the number of mucosal mast cells, eosinophils or globule leucocytes. They remain largely inconclusive (Paolini *et al.*, 2003a,b ; Rios *et al.*, 2008; Tzamaloukas *et al.*, 2006).

Based on the hypothesis that tannins might affect the nematode according to a pharmacological process, some recent insights have been obtained on the interactions between these polyphenolic compounds and the infective larvae, using sainfoin as a model of TR fodder. Both *in vitro* and *in vivo* data indicate that the presence of tannins disturb the two early steps of nematode establishment, first the larval exsheathment (Brunet and Hoste, 2006, Brunet *et al.*, 2007) and second, the penetration of the exsheated larvae within the digestive mucosae (Brunet *et al.*, 2008). These functional modifications have been associated with major changes in the larval ultrastructure. Similarly, observations in Transmission and Scanning electron microscopy on adult *H. contortus*, after *in vitro* and/or *in vivo* contact with TR plants have shown major modifications to the cuticle, the digestive tract and the female reproductive tract which can explain the negative consequences on adult worm populations, and particularly those affecting the egg excretion. However, the exact mechanisms of action remain obscure and could differ depending on the parasite, its stage of development and possibly the biochemical characteristics of forage species. Despite the recent progresses, further studies remain necessary to better understand how tannins and other flavonoids affect the different stages of parasitic Nematodes.

From the *in vivo* results, the consumption of TR forages by sheep or goats has been associated with a modulation of the nematode biology explained by impacts on different key stages of the life cycle (Figure 1).

VI – Conclusion

The studies performed on the AH effect of TR forages have underlined their potential value as nutraceuticals to prevent or limit parasitic infections of the digestive tract. The results suggest that the impacts on the nematode biology are probably multiple, by affecting different key stages of the life cycle. The combination of these effects contributes to slow down the dynamics of infection. The overall effect on host resilience described in many studies is also a positive aspect associated with the consumption of these TR Legumes. Due to the need to adapt to different agronomical constraints, several bioactive TR forages have now been identified and used under various environmental conditions. However, these studies have also illustrated some of the current limits in the use of TR legumes as nutraceuticals. Pending questions remain to be solved to permit a more pertinent use in farm conditions. In particular, answers to the questions related to the modes of action of tannins on parasites appears essential to acquire. Because of the worldwide distribution of both .gastrointestinal Nematodes and of TR plants belonging to a wide range of botanical families, the field of potential application is particularly large, as far as the mechanisms of action will be understood. To this respect, TR legume forages appear as valuable models of study.

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