IDENTIFICATION AND VALIDATION OF BIOACTIVE PLANTS FOR THE CONTROL OF GASTROINTESTINAL NEMATODES IN SMALL RUMINANTS

HERVÉ HOSTE[2] JUAN FELIPE TORRES-ACOSTA[1],
MIGUEL ÁNGEL ALONSO-DIAZ[1], SÉVERINE BRUNET[2],
CARLOS SANDOVAL-CASTRO[1] and SYLVIE HOUZANGBE ADOTE[3]

[1]FMVZ, Universidad Autónoma de Yucatán, Mérida, Yucatán, México.
[2]UMR 1225 INRA DGER, 23 Chemin des Capelles, F31076, Toulouse, France.
[3]Faculté des Sciences Agronomiques, Université d'Abomey Calavi, 01 BP 526 Cotonou République du Bénin.

Abstract. The search for bioactive plants which can be used as non-conventional anthelmintics (AHs) has received considerable attention in recent times because of the increasing, worldwide development of resistance to chemical anthelmintics in worm populations. However, scientific evidence to validate the use of plants remains limited. The criteria required for the scientific validation of phytomedicine and nutraceuticals are similar to those expected for chemical anthelmintics: definition and quality, efficacy, identification of side-effects. However, the methods of investigation need to take into account i) the variability in bioactive compounds in the natural resources, ii) the mode of distribution to animals and iii) the potential anti nutritional and side-effects of some bioactive compounds and the possible occurrence of regulative mechanisms in the hosts. These points are critically discussed, in particular by reference to the example of tannin rich plants with anthelmintic properties from both temperate and tropical regions.

INTRODUCTION

Gastrointestinal nematodes (GINs) remain worldwide a major threat on the outdoor breeding of small ruminants. Because of their large geographical distribution and of the severe pathological consequences that they provoke, GINs are responsible for major economical losses in sheep and goats under the different latitudes. For more than 50 years, from phenothiazine to the recent amino-acetonitrile derivates, the pharmaceutical industry has offered to the veterinarians and farmers the chemical ammunitions making feasible an efficient control of GINs. However, despite constant inputs in research and development of new chemical drugs, plant and plant products remain largely used worldwide. Moreover, since the last 15 years, the interest for these natural resources has received a strong impetus because they represent a novel approach to control GINs. Three main considerations can be identified which explain this renewed interest in plants with anthelmintic (AH) properties:

1. *In many developing countries, the difficulty for small farmers to access to AH drugs and to afford their cost.* In contrast, the traditional, local resources are of low cost, easily available and their potential use has been acknowledged and recommended for centuries over the five continents.

2. *In developed countries, and in some population strata of the developing countries, the increasing public concern on the use of chemical drugs in farming industry and on the potential impact on animal food products and on the environment.* This lead to the current trends for the development of production systems which are more environmentally friendly, aiming at reducing or even at eliminating the reliance on chemical inputs. In such systems, like Organic Farming or production with signs of quality, the need for natural substances to complement or to replace the chemical drugs is a real issue.

3. *Since the early 70' of the last century, the constant increasing development of AH resistances in worm populations*. The partial or total failure of GIN control by use of chemical drugs is nowadays largely acknowledged (Jackson & Coop 2000, Kaplan *et al.,* 2004). This worldwide phenomenon explains the current seek for, more sustainable, alternative solutions, adapted to the various systems of breeding sheep and goats. The use of natural resources is one of these alternative solutions currently explored.

Last it is worth to underline that WHO estimates than throughout the world, more than 70 % of the drugs used for a therapeutic goal are from natural origin. Moreover, 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)

In the present paper, we aim at presenting critically

1) The different approaches which can be applied to identify natural (plant) resources for the integrated control of GINs.

2) The methodologies available to validate on scientific grounds the potential AH properties of these plant or plant products and their efficacy against GINs in sheep and goats

3) The balance between the positive and the negative effects of these natural AHs and the limits inherent to the use of these natural resources

4) The questions leading to future research priorities.

Because of the large field addressed, of the current abundance of literature and the exponential number of articles referring to the subject, this review cannot be extensive. Hence, in order to illustrate some of the previous items and future questions to be addressed, we took the option to mainly restrict our choices of references to the example of tannin rich (TR) plants, both in temperate and tropical conditions. However, it is expected that this approach might be worth to be applied to other natural resources, in particular some of the most largely distributed and studied tropical plants [e.g.. neem *(Azadirachta indica*) (Chandrawathani *et al.*, 2002, 2006; Costa *et al.*, 2006, Hordergen *et al.*, 2003; Neem website, 2001) or papaya (Satrija *et al.*, 1995, Stepek *et al.*, 2004, Hounzangbe-Adote *et al.*, 2005a,b)] which are currently under investigation and for which tannins are not suspected to be the main active components.

HOW TO IDENTIFY NATURAL RESOURCES WITH POTENTIAL AH PROPERTIES?

Random screening

A first way to identify potential resources with biological activity is based on the general screening of a majority of plants representative of a same geographic / climatic area. To be time and cost effective, such method requests the use of large scale automatized screening techniques, applied to well-identified, standardised and meaningful target models such as different stages of pathogens, culture of specific cell types, etc... There are a few examples of such methodology being applied based on large public or private fundings (Gordon *et al.*, 2007; David, 2006). A possible disadvantage is that the results obtained with a certain screening technique or on a specific target model might not be extrapolated to real *in vivo* situations. It also might overlook other possible substances with AH activity, contained in the plant, but that the screening technique is not able to identify. or extract, therefore these are not tested. However, in most cases, the mode of investigation of plant properties tries to be much more selective as a starting point. Three main modes of discrimination can be described as possible filters.

Exploitation of previous knowledge on the biochemical nature of the bioactive compounds

It is usually considered that plant secondary metabolites (PSMs), i.e. metabolites not directly involved in plant growth or reproduction, like tannins, alkaloids, glycosides, terpenes, … are involved in the AH properties of various plants throughout the world. Consequently, one efficient way for selective screening of natural resources with antiparasitic properties is based on the identification of the active compounds involved in the AH activity of a few plant models. Thereafter, this led to the possible screening of plants from different botanical families but which share similar PSM components. This scientifically sounded approach has been applied for tropical plants rich in proteases (Stepek *et al.*, 2004) or for temperate and tropical plants rich in condensed tannins (Hoste *et al.*, 2006). Starting from tannin rich (TR) temperate legume forages initially identified with AH properties (Niezen *et al.*, 1995,1998a and b), the interest is nowadays being extended towards temperate (Osoro *et al.* 2007a and b) or tropical (Alonso-Diaz *et al.*, 2008, Minho *et al.*, 2007, Cenci *et al.*, 2006, Kahiya *et al.*, 2003, Nguyen *et al*, 2005) TR fodders, bushes or trees. In the latter, the complexity and the variability of the tannin molecules makes difficult to identify the active compound. Usually, they are broadly described as condensed tannins.

Exploitation of traditional knowledge = Scientific validation of traditional resources

The interest for traditional knowledge on the therapeutic potentials represented by local resources is receiving a growing attention on all the continents (Akhtar *et al.*, 2000, Hammond *et al.*, 1997, Dichl *et al.*, 2004, Guarrera 1999, Kozan *et al.*, 2006, Waller *et al.*, 2001). Methods based on questionnaire surveys focussed on end users of this "technology" have been nowadays validated in order to select the best potential hits from the ethnoveterinary pharmacopea and for further validation by *in vitro* and *in vivo* methodologies (Houzangbe-Adote 2004). These methods are by essence multidisciplinary since being at the interfaces between veterinary medicine, biochemistry, botany, anthropology and sociology. They request skilled assistance from experts of all these disciplines.

Exploitation of animal knowledge on self medication

The first indication relevant to this aspect have been acquired from primates, like chimpanzees or bonobo (Huffman,1997). The approach has led to the discovery of new drugs to treat malaria and to their current scientific validation (Krief *et al.,* 2004).Evidence of the abilities for self medication in small ruminants has not yet been clearly described, although the occurrence of such processes has been suggested (Provenza, 2003). However, the study of self medication in ruminants needs to clearly differentiate between social, evolutive, adaptive and nutritional trade-off effects to those of the sole apparent intention of a medicinal effect in animals.

HOW TO USE THE NATURAL RESOURCES WITH AH PROPERTIES?

In veterinary medicine, two modes of application of plants can be distinguished i.e. nutraceuticals *vs* medicinal plants (= herbal or phytotherapeutic drugs /products). This distinction mainly relates to the mode of use although it has also consequences for the characterization of the resources, the scientific validation of the efficacy against GINs, the assessment of potential negative effects. This difference might also have consequences on the regulation rules which would apply for both types of products. Whereas the definition of nutraceuticals might be assimilated to those of food or food additive, the second concept (herbal drug) is by nature closer to a medicinal commercial drug.

Nutraceuticals

These are forages or browsed plants whose traditional exploitation in farm conditions was initially associated with animal feeding,because of their nutritive value but which are nowadays also considered for their beneficial effects on animal health because of recent results illustrating these other potential advantages. The mode of use of nutraceuticals remains those of a feed source, either fresh or conserved, i.e. hay or silage. In the last decades, the best examples of this concept of nutraceuticals in ruminants have been provided by TR legume forages, like sulla, sericea lespedeza or sainfoin (cf Table 1). However, recent results on a few examples of TR browse or fodders exploited in tropical countries, e.g. *Leucaena leucocephala* (Ademola *et al.*, 2005, 2006), various species of *Acacia* spp (Kahiya *et al.*, 2003; Cenci *et al.*, 2006; Minho *et al.*, 2007), cassava (*Manihot esculenta*) (Sokerya & Rodriguez, 2001; Sokerya & Preston, 2003; Nguyen *et al.*, 2005) have also been acquired.

Nutraceuticals can be used on a long-term scale trying to combine their nutritive and sanitary value in order to prevent clinical outbreaks of nematode infections, to limit the epidemiology of GINs and to reduce the reliance on chemical AHs for the control of these parasitic diseases. Due to the mode of distribution, when using nutraceuticals, it is necessary to confirm that animals are willing to eat enough quantity of plant materials for a sufficient time to obtain a preventive AH effect. Last, it is important to identify the economic threshold between the AH effect and the potential anti-nutritional effects associated with the exploitation of these plants.

Phytotherapeutic drugs

These are preparations of plants or of plant extracts whose administration to animals is restricted to a short term period and which first aimed at treating infected animals (curative effects). In contrast to nutraceuticals, traditional herbal remedies or plant drugs have first been dedicated for long to therapeutic use in domestic animals. Like nutraceuticals, their properties on animal health have usually been associated with their content in plant secondary metabolites (PSM). In many cases, these herbal remedies represent a mixture of plants or plant extracts obtained through various physical or chemical processes. A phytotherapic drug is usually composed with 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 it has consequences in the definition of the products and validation of the efficacy.

HOW TO CHARACTERISE THE NATURAL PRODUCTS AND THEIR POTENTIAL AH PROPERTIES?

Because plants or plant products are used for their AH properties in order to prevent or treat parasitic diseases, the end users (farmers, veterinarian) and the consumers can expect that a range of criteria similar to those applied for chemical AHs (i.e; efficiency, definition and quality of products, negative side effects) will be described when using plant remedies or nutraceuticals. However, the natural origin of the antiparasitic resources has consequences on the interpretation and appreciation of these criteria. We will use the comparison with the expectations on the chemical drugs to illustrate some limits shown by plants or plant products to control GINs . However, to interpret such a comparison, it is important to underline that the objectives associated with the use of a plant AH might slightly differ from those assigned to chemical drugs (Ketzis *et al*, 2006).

Definition and qualitative characterisation of the natural products

Chemical AH are well defined products. The active substance is usually unique and standardised, making the chemical drug available with the same quality worldwide. In addition, methods of identification and of measurements of the active substances are described.

In contrast to chemical AHs, nutraceuticals and herbal medicines are not well-defined products. As previously stated, PSMs are usually suspected to play a main role in the

Table 1. Examples of models to assess the AH efficacy of TR fodders used as nutraceuticals in sheep (S) or goats (G)

	In vitro test	*In vivo* experimental rodent models	*in vivo* controlled indoors conditions	*in vivo* field and farm studies
Sulla	*Molan et al 2000, 2002*		*Pomroy and Adlington 2006 (G)*	*Athanasiadou et al 2005 (S)* *Tzamaloukas et al, 2006 (S)* *Niezen et al 1995; 1998 (S)* *Tzamaloukas et al, 2005 (S)*
Sainfoin	*Molan et al 2000, 2002* *Paolini et al, 2004*		*Paolini et al 2005 (G)* *Heckendorn et al 2006, 2007(S)*	*Hoste et al, 2005 (G)* *Paolini et al 2005 (G)* *Athanasiadou et al 2005 (S)*
Sericea lespedeza			*Shaik et al 2004, 2006 (G)* *Terril et al 2007 (G)* *Lange et al 2006 (S)*	*Min et al, 2004* *Lange et al 2006 (S)*
Lotus corniculatus L. pedunculatus	*Molan et al 2000, 2002*		*Heckendorn et al 2007 (S)* *Niezen el al 1998, 2002 (S)*	*Athanasiadou et al 2005 (S)* *Bernes et al, 2000 (S)* *Marley et al, 2003 (S)* *Niezen et al 1998, 2002 (S)* *Tzamaloukas et al, 2005 (S)* *Ramirez Restrepo et al 2004, 2005 (S)*
Chicory			*Heckendorn et al 2007 (S)* *Tzamaloukas et al, 2006 (S)*	*Athanasiadou et al 2005(S)* *Marley et al, 2003(S)* *Tzamaloukas et al, 2005 (S)*
Cassava			*Sokerya and Preston, 2003* *Sokerya and Rodriguez, 2001* *Nguyen et al, 2003, 2005 (G)*	
Leucaena leucocephala	*Alonso Diaz et al 2007a and b* *Ademola et al, 2005,2006* *Lopez et al 2004*	*Rojas et al, 2006*	*Rojas et al, 2006 (S)* *Nguyen et al 2003, 2005 (G)*	

antiparasitic activity of a plant or a plant remedy. However, the exact nature of the active compounds involved is not always known. Moreover, plants usually contain several potentially active PSMs as it has been shown for TR legumes from both temperate or tropical areas. In addition, it is known that for the same plant species, the PSM content is highly variable in quantity and quality, due to genetic and/or environmental effects (Makkar *et al*., 2006). Some examples of genetical effects are the location of the PSM's in the plant tissue and parts, cultivars and the rate of change due to maturity of the plant. Examples of environmental effects are soil conditions, water and light availability, harsh environmental conditions, degree of predation; mode of conservation. Another source of variations relates to the mode of physical or chemical preparation of the extracts involved in the composition of plant drugs (extraction, decoction, expression, infusion, etc…;). The same criticisms apply for the preparation of essential oils which form the basis of aromatherapy (Lahlou, 2004).

Despite these inherent difficulties related to variations in plant composition, it is possible to define criteria which should be mentioned in order to characterise as precisely as possible the material used. The exploitation of non standardised natural resources imposes first to describe in details the way by which the resources have been selected, identified and treated in order to obtain the plants, plant products or plant extracts whose AH activity is measured. To this respect, the criteria recommended in the Guidelines summarising the rules on the Human Pharmacopea and emitted either by the European or the US Pharmacopea committee in order to improve the definition and the conditions of use of herbal products when plants are aimed for medicinal properties (EMEA Guideline on declaration of herbal substances and preparations) represent a first valuable approach. These recommendations compose a key scientific requirement before any validation of antiparasitic activity in order to make the assessment of efficacy reproducible.

The different technological steps and criteria desirable for the characterisation of plant resources before examination for bioactivity are:

1. Conditions of collection (Where? When? Which plant stages? Which parts of plants? etc…)
2. References on the criteria for identification of the plants
3. Conditions of standardised storage and of stable preservation of the samples
4. Description of conditions for standardised extraction before bioguided assay
5. Conditions of fractionation if applied
6. Detailed methodology of structural analysis: (e.g. HPLC, mass spectrometry, nuclear magnetic ressonance).

Points 5 and 6 are compulsary only in the case of a process aiming at the identification and / or isolation plus analytical characterisation of the active compounds.

Besides, methods of measurements of the active compounds should also be notified. This complexity in the biochemical characterisation of the material exploited is sometimes reflected in the diversity of methods of measurement available. This is illustrated with the example of tannin rich plants. The methods of measurement of the active compounds are not strictly standardised and there is a need of a harmonization of methods applied in the different laboratories. For tannins, the current trend is to request detailed description of the methods of chemical measurements for total phenols, total tannins, hydrolysable tannins and condensed tannins together with a detailed description of the standard used, combined with methods enabling to evaluate the biological activity of tannins (i.e ability to bind to proteins) (Makkar, 2006, Mueller-Harvey, 2006). Therefore, fast, inexpensive, replicable and accurate methods of measurement of the active compounds should be developed.

When not directly available, due to the complexity of methodology to measure the active compounds, another possible approach is to use techniques which quantitatively measure biochemical / physical markers which are directly correlated with the components of interest. An example of such biochemical approach is the measurement of the urease activity of soybean as a marker of its trypsine inhibitors. An example of the physical approach is the production and persistence of foam from a saponine containing material.

Efficacy

For any AH, either chemical or natural, efficacy is the first criteria to be fulfilled. However, by many aspects, the results obtained with plant AHs differed from the expectation of high (>: 95 %) efficacy and broad spectrum which is usually achieved with conventional chemical drugs. For example, with TR plants, most of the current data suggest that values of 60 to 80% are among the highest reductions observed for parasite egg excretion (Paolini *et al.*, 2003a, 2003c,; Shaik *et al.*, 2004, 2006; Heckendorn *et al.*, 2006, 2007; Terrill *et al.*, 2007), and for worm numbers or female worm fecundity (Paolini *et al.*, 2003b & 2003c, Heckendorn *et al.*, 2007, Terrill *et al.*, 2007) when animals consumed TR forages. In addition, several results have illustrated the fact that the efficiency against the different nematode species varies, in particular when considering species inhabiting separate digestive organs (Athanasiadou *et al.*, 2001, Hoste *et al.*, 2006, Heckendorn *et al.*, 2007).

There is no current specific Guidelines to test the antiparasitic activity of natural or plant AHs. In this context, the recommendations and methodologies described in the WAAVP guidelines (Wood *et al.*, 1995) which have been developed to assess the AH efficacy of chemical drugs, remain the most valid approach to determine the activity of plants or plant products, although a few adaptations have yet been proposed (Lorimer *et al.* 1996).

In vitro tests

In vitro assays present several advantages. They are usually simple to perform, of a relative low cost, are reproducible, require a limited amount of biological material and usually, with the exception of AMI, do not imply the slaughtering of animals. They target either different parasitic stage, ie, the eggs (egg hatchings or development [Egg Hatch Assay (EHA) or Larval Development Assay (LDA)], as well as the biology of free living larvae(larval feeding inhibition assay (LFIA), or third stage infective larvae (larval migration inhibition assay (LMIA), larval exsheatment assay (LEA)] or last, the adult worms, through the motility of adult worms (AMI) (Gordon *et al.*, 2007). They have usually been directly applied on the main nematode species parasite of ruminants, because in many instances, the relevance of models of free living nematode models such as *Caenorhabditis elegans* is questioned (Geary & Thompson, 2001).

It is worth also to compare the effects of a same extract or compound on two different nematode species, if possible one abomasal and one intestinal one, in order to address the question of the specificity of activity. As mentioned previously, differences in effects depending on nematode species have been reported in several studies on tanniniferous plants but it has not been determined yet whether it is due to a difference in exposition of worms to various concentrations of the active compounds along the digestive tract or whether this reflects relative differences in susceptibility to natural compounds. *In vitro* assays on different species provide a mean to solve the question.

In a few studies, information and /or confirmation of the specific role of some particular PSM in the AH activity has been provided by the addition to the extracts of specific inhibitors for the suspected bioactive compounds. For example, two inhibitors of tannins, PVPP or PEG, have largely been used to examine the role of tannins in the AH properties of plants form diverse botanical origins. However, the lack of strict specificity of these tannin inhibitors has been questioned on a few occasions (Barrau *et al.,* 2005) but this is frequently overseen.

One of the main disadvantages of the *in vitro* assays is the difficulty to interpret the results because of the major differences occurring between *in vitro* and *in vivo* conditions. Even if the question of the significance of the extract concentrations applied *in vitro* is addressed by comparison to what is known *in vivo*, still considerable physiological changes might be encountered in animals. The latter is in regard of the possible degradations or transformations of the active compounds along the digestive tract and their possible interactions with other components of the luminal environment, like fibre or proteins, or other PSMs.

In vivo tests

In vitro assays are particularly useful for the initial screening of the activity of plant extracts or of biochemical fractions in order to select those worth to be further examined. However, the conclusions gained from the *in vitro* results always have to be validated by further *in vivo* studies both in controlled and in farm conditions. As previously stated, the general WAAVP recommendations and guidelines on anthelmintics (Wood *et al.,* 1995) represent a valuable approach although some desirable adaptations have been yet suggested such as to increase the number of animals per group because of the usual lower efficacy of plant AH when compared to chemical drugs (Githiori *et al.*, 2006). Due to the variability in plant efficacy, depending on the parasite species, it seems crucial to address the question of the specificity of effects by seeking information on a range of nematode species inhabiting different parts of the digestive tract.

Experimental rodent models present the advantages to permit higher number of animals per group and thus to make easier the determination of the dose to be applied *in vivo* in order to confirm the antiparasitic activity suspected according to the *in vitro* results. For example, infections of mice with *Heligmosomoides polygyrus*; rats with *Nippostrongylus brasiliensis,* or gerbils with *Haemonchus contortus* (Satrija *et al.*, 1995; Githiori *et al.*, 2003; Rojas *et al.* 2006) are among the experimental models which have been largely used and which have provided information on the *in vivo* efficacy of a range of plants from various botanical origins.

However, for most PSMs, the host physiological conditions, in particular the ruminal conditions and the interactions with the ruminal particles, can strongly influence the availability of free PSMs to interact with the worms and to affect their biology. Therefore, direct confirmation of the results on small ruminants seem to be a requisite when studying natural AHs. However, because plants, in contrast with chemical drugs, are not well defined products, and because they are distributed as feed, the dose received by the animals can be modified by its voluntary feed intake (VFI). Therefore, several additional specific questions should be considered in the design of *in vivo* assays when testing plant dewormer efficiency. Some of those are listed herein

- How to feed/dose the animals with the nutraceutical and/or the phytotherapeutical product?
- How to measure the consumption of the nutraceutical by the animals?
- Quality and variability of the plant material used (with or without conservation)?
- Do the animals have a previous feeding experience with the plant material assayed?

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Also, it seems important to define the potential interaction with other PSM's that can result in the modification of the expected intake (Rogosic *et al.,* 2006). The same might be true for feed components such as protein which can link with tannins (Makkar *et al.*, 2007; Mueller-Harvey, 2006), thus possibly reducing the expected AH effect. Moreover, because some plants selected for bioactivity also present some nutritive value, it is important to take into account any possible confounding effects related to an improved immune response of the treated animals because of a better coverage of nutritional requirements (Coop & Kyriazakis, 2001).

Last, evidence is accumulating to suggest that the effect of natural AHs provoke more a modulation of worm biology than a real nematodicidal effect. Therefore, the main impact might be through a modulation of epidemiology of infection. Consequently, it sounds logical to eventually address the question of the efficiency of these plant AHs in farm or field studies. In the case of tanniniferous legume forages like sulla, sainfoin, or sericea lespedezea, data have been produced (see Table 1 for references). For browse legumes used as nutraceuticals, the references are much less frequent (Kabasa *et al.*, 2000). Last, in the case of traditional plants, those sort of studies mimicking field conditions remain scarce and they present difficulties to full fill the conditions of experimental design and of statistical interpretation of the results (Hounzangbe-Adote *et al.*, 2005c) However, such an approach seems essential to disseminate back the results towards the small scale farmers. This approach forces to take into account and to report on the constraints imposed either by the collection of the plants from fields or by the need of preservation for the right time/ period of distribution to animals in regard of the epidemiological conditions for parasite infections.

Mechanism of action

To understand the mode of action of AHs is a key requirement for a pertinent use of any drug in farm conditions. For the chemical AHs, knowledge on the nature of the active compound, on the mechanisms of action against the nematodes and on on its pharmacology in the host are generally identified. In addition, one (or only a few numbers) of standardised chemical substances are incriminated for the AH properties. In the current state of art, most of this information is usually lacking for plants and plant products proposed as natural AHs.

In many cases, the plants selected for their therapeutic properties in traditional veterinary medicine share common indications with human medicine (Houzangbe-Adote, 2004). Moreover, nutraceuticals have been used for long as a feed resource. Therefore, qualitative information on their major constituents, sometimes based on recent methodologies of biochemical analysis is usually available (Fennel *et al.*, 2004). On the other hand, for a same plant, the biochemical composition in quantity and proportion of compounds might vary according to several factors and quantitative information aiming at measuring this variability is usually lacking. Similarly, in the case of AH efficacy against nematodes of ruminants, the identification of the role played by a few main components is usually absent or incomplete. Last, plants usually represent a "cocktail" of different PSMs, which might possess different degrees of AH activity and the hypothesis of detrimental, additional or synergystic interactions between the different compounds can not be discarded although they remain difficult to demonstrate.

For example, evidence begins to accumulate indicating that condensed tannins play a central role in the activity of TR legumes forages or browse against GINs (Hoste *et al.*, 2006). However the hypothesis of differences in AH activity depending on the structure and nature of polymers (e.g. between the 4 biochemical classes of condensed tannins) has now been proposed and, to some extent, substantiated (Molan *et al.*, 2003, Brunet & Hoste, 2006, Brunet *et al.*, 2007, 2008). In addition, the possible involvement in the AH activity of flavonoids which are also found in many legume forage ahs also been evoked (Barrau *et al.*, 2005). Similarly, in the case of chicory (*Cichorium intybus*), another temperate fodder with AH properties (Table 1), a main role for sesquiterpene lactones has been incriminated because they represent the major constituents of the plant although the presence and activity of a small amount of condensed tannins remain questioned (Marley *et al.,* 2003, Tzamaloukas *et al.*, 2005, 2006).

On the other hand, information on the mode of action of the plant extract and /or suspected active compounds on the different stages and/or species of nematodes is usually absent. A few studies have started to address this central question of the mechanisms of action of plant components (for tanniniferous forages, see Brunet *et al.*, 2006, 2007, 2008; for plants rich in cysteine proteinases like papaya, pineapple or fig, see Stepek *et al.*, 2004) but they remain an exception. Last, information on the metabolization of the active compounds (i.e. their degradation / transformation, possible binding with digestive particles and absorption in the different parts of the digestive tract) which conditioned the exposure of worms to the active components remain largely speculative.

Negative side- effects

Natural does not mean safe

Because of their long usage to treat animals, plants used in traditional medicine are assumed to be safe. Similarly, because they have been used for centuries as feed resources, nutraceuticals are usually considered as being without toxic effects. However, potential hazards associated with long term effects of plant can occur. In addition, the possibilities of misidentification of plants, mistakes in preparation and/or administration can not be discarded. Therefore, like for chemical AHs, the question of potential toxicity of plant extracts or fractions is worth to be examined although some specificities in the use of plant AHs have to be taken into account. In a first step, to evaluate the possible acute toxicity of natural compounds**,** *in vitro* models exist either on cultured cell lines or against invertebrates like brine shrimp larvae (Fennell *et al.*, 2004, Mc Gaw *et al.*, 2007). In a second step, *in vivo* models, including experimental ones, can also be exploited to confirm suspicion based on *in vitro* data (e.g. Camurça Vasconcelos *et al.*, 2004).

Only the dose level makes a poison. (« Tout est poison, rien n'est sans poison. Seule la dose fait qu'une chose n'est pas un poison ! »).

This major concept enounced by Paracelse in the 15th century applies both for chemical and natural AHs. Examples of a balance between the positive (efficacy) and the negative effects (potential direct toxicity), depending on the dose, are well known and have been largely substantiated for most of the current broad spectrum AHs. For any chemical drug registration, these aspects are investigated and information provided. In particular, the definition of a safety coefficient i.e. the ratio between the therapeutic and the toxic dose is requested.

Because most PSMs participate in the plant defense against predators, like insects or herbivores (Jean-Blain, 1998), dose-dependent negative consequences on digestive physiology have frequently been described both in monogastrics and in ruminants (Perevolotsky, *et al.*, 2006). Several studies have shown that above a certain threshold of condensed tannins in the diet, they exert some negative effects by limiting the consumption of plants by the host and provoking negative effects on digestibility (Athanasiadou *et al.* 2001; Ramirez - Restrepo *et al.*., 2005). For TR legume forages, values above 6 to 7% of CT in the DM are usually mentioned as the threshold value for these negative effects whereas AH properties have been described at a lower range of 2.5 to 6%. For tropical legume browse, information on these topics remains less clear. Several data also suggest that these threshold values might also depend on **i)** the nature and biological activity of tannins in the respective plant material, **ii)** the ruminant species, depending whether they can be classified as full grazers, intermediate browsers or full browser, **iii)** adaptation of the ruminal microbiota, **iv)** experience of the animal fed with the plant material and **v)** interactions with other PSM's or antinutritional factors such as lignin (Alonso-Diaz *et al*., 2007a,b).

Possible regulation of ingestion in ruminants/ Physiological adaptation to PSMs

One of the main difference between the conventional and the natural AHs relates to the mode of administration. For chemical drugs, the posology is pre determined by the pharmaceutical company. If respecting the practical recommendations of use, the dose is imposed to the animals by the farmer or the veterinarian. In contrast, particularly when used as nutraceuticals, plants are part of the animal feed. Therefore, the host feeding behaviour and experience with the feed might contribute to limit the plant voluntary ingestion (VFI) and consequently to reduce the associated effects either negative or positive. For example, for TR plants, regulative mechanisms, like the occurrence of post-ingestive negative feedback (Provenza, 2003) have been identified which limit the negative effects of tannins by reducing ingestion. This explains why a period of adaptation to the feed seems necessary to examine the possible antiparasitic effects of a plant and why measurements of refusals is a key requirement.

In addition, for some PSMs, like tannins, adaptive physiological mechanisms have been described at different levels, (eg. saliva, gastrointestinal flora, detoxification pathways which contribute to limit the possible negative effects of the compounds. In herbivores, the ability to develop such adaptations seems to differ depending whether the animals are strict grazer, intermediate or full browsers (Robbins *et al.*, 1991, Silanikove, 2000). On the other hand, the question of a possible interference of such mechanisms with the antiparasitic properties should be addressed (Alonso Diaz *et al.*, 2007 a and b). Such differences in adaptation to PSMs have been observed between sheep, goats and deer (Silanikove *et al.*, 1996). They might explain some variations in antiparasitic results observed between ruminant species although this point needs further investigation (Hoste *et al.*, 2006).

CONCLUSIONS AND FUTURE DIRECTIONS FOR RESEARCH

Since the last decade, the number of studies dedicated to the scientific validation of the antiparasitic effects of plants has shown an exponential growth. For some plant products or some bioactive compounds, like condensed tannins, evidence has accumulated indicating that plants whose AH properties have been to some extent scientifically validated, might represent a valuable solution within the "basket of options" (Krecek & Waller, 2006) to achieve a more sustainable control of GINs with a reduced reliance on chemical AHs. However, whatever the herbal remedies or nutraceuticals to be exploited, at least four main directions for future research can be foreseen to make possible this implementation.

To avoid an empirical use and instead to promote a pertinent exploitation of the plant resources with AH properties by the end-users supposes a better understanding of the mode of action on the worms and of the factors which can explain the origin of variability in results which has been reported with different plant medicines.

- A first aim is to better understand the factors which affect the plant content in PSMs and their bioactivity. This implies first a better standardisation of and an agreement on the methodologies for the preparation of plants and plant extracts and for the identification and measurement of the active components responsible for the bioactivity. For this prospect, the creation of an *ad hoc* committee to finalise Guidelines to examine and validate the AH efficacy of natural resources would constitute a first step to target.

- A better understanding of the mechanisms of action of bioactive PSMs on the worms and of the interactions with the elements composing the digestive luminal contents, in particular nutrient component, is also a key requirement. The current lack of information on these aspects is striking.

- For a long term use of AH plant products and for an efficient implementation of a sustainable control of GINs with these approaches, a better understanding of the factors governing and of the mechanisms underlying the relationships between plants, PSMs, small ruminants and nematodes and the balance between the positive and potential negative effects of PSMs is required. In particular, the question of the possible trade-offs between the positive and the negative effects of PSM and how the presence of gastro-intestinal nematodes in ruminants modulate these trade offs represent main fields of research.

- Last, it seems also essential to increase the number of field studies in various epidemiological conditions, to assess the potential of plant resources for an integrated approach of GIN parasitism. This is not only important to evaluate the economic balance of a higher reliance on natural local resources, but also to take into account the agronomical, botanical, ecological and sociological implications of such options. A holistic approach supported by the participation of specialists from a large range of scientific fields is highly desirable to help small farmers to contain nematode parasite infections in sheep and goats by relying on natural resources.

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