



I FORUM SOBRE USO DE PLANTAS TANINÍFERAS NO CONTROLE DE HELMINTOS EM RUMINANTES

Local: Centro de Energia Nuclear na Agricultura, USP, Piracicaba, SP

Período: 17 e 18 março de 2008

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PROGRAMA

I FORUM ON THE USE OF TANINIFEROUS PLANTS ON CONTROL OF HELMINTHS OF RUMINANTS

17/03/2008 - Monday

| | |
|--------|---|
| 9hs | Open Section |
| 9h30m | Taniniferous plants and animal nutrition Prof. Dr. Sobhy Sallan (CENA-USP-SP) |
| 10h30m | Coffee break |
| 11hs | Brazilian Sources of Tannins Prof. Dr. Adibe L. Abdalla (CENA-USP, SP) |
| 12hs | Lunch |
| 14hs | Taniniferous plants and helminthes control – sheep Dr. Frank Jackson (Moredun Research Institute – Edinburgh, Scotland) |
| 15h30m | Coffee break |
| 16hs | Taniniferous plants and helminthes control – goats Dr. Hervé Hoste (INRI – Toulouse - France) |
| 17h30m | Forum Dinner |

18/03/2008 - Tuesday

| | |
|--------|---|
| 9hs | Methods of analysis of tanniniferous plants Dr. Frank Jackson (Moredun Research Institute – Edinburgh), and Prof. Dr. Adibe L. Abdalla (CENA-USP) |
| 10h45m | Coffee break |
| 11hs | Oral presentation (10 minutes each presentation and 5 minutes questions) |
| 12h30m | Lunch |
| 14hs | Oral presentation (10 minutes each presentation and 5 minutes questions) |
| 15h30m | Coffee Break |
| 16hs | Discussions with groups involved in projects with tannin and control of helminthes – organize a multi group Project. |
| 18hs | Closed Section |

EFFECTS OF TANNINIFEROUS PLANTS ON NEMATODES IN SHEEP AND GOATS

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The search for alternative solutions to chemical treatments has been prompted by the rapid emergence and the widespread diffusion of resistance to anthelmintics in populations of gastrointestinal nematodes in sheep and goats. Experimental evidence nowadays accumulate to suggest that tannin rich (TR) plants possess anthelmintic properties and thus that they may represent an alternative option for the control of nematodes.

The first results were acquired in sheep. However, several reasons explain the interest of specific studies in goats: i) the high level of resistance to anthelmintics in goats, which impose an urgent seek for alternative solution; ii) the possible use of goats as a model for the use of TR plants in dairy small ruminants; iii) goat is usually described as a “browser” or “intermediate browser” species compared to sheep, which is a “grazer”. This supposes the existence of possible behavioural and/or physiological adaptations for the exploitation of TR plants which might modulate not only the possible toxicity of these compounds, but also the potential efficiency against nematodes of the digestive tract.

Several in vivo studies performed in goats using TR plants of different categories [TR forages such as sainfoin (*Onobrychis viciifoliae*) or sericea lespedeza; TR plants composing the browse of Mediterranean or tropical areas] have confirmed the AH activity of tanniniferous plants against the infective larvae and the adult worms of the principal nematode species. The main effects

on the infective, third stage larvae were a reduction of larval establishment. A reduction in female worm fertility, sometimes combined with a reduction in worm number, has been the main consequences observed on adult worm populations explaining the significant decrease in egg excretion repeatedly found in various studies. The use of specific inhibitors of tannins confirms the role of these polyphenolic compounds in the observed activity. Condensed tannins seem particularly involved. However, variations in results occur depending on several factors related to the plant, the nematode or the host.

1) The concentration and the nature of tannins in the diet are two of the main factors to be controlled to increase the efficiency of bioactive plants against nematodes. In both small ruminants species, a minimal concentration in diet above 2,5 to 3,0 % seems required to observe antiparasitic effects. On the other hand, a few evidence suggest that goats might tolerate higher concentrations of tannins in diet than sheep.

2) A better understanding of the mode of action of tannins against the nematode is required to analyze the origin of variations depending on the worm species and/or stage and to permit consequently, a more pertinent application of these nutraceuticals in farm conditions. Results obtained from both in vitro and in vivo studies illustrate the effects of tanniniferous plants on the 2 successive biological processes which compose the initial step of host infection, i.e the establishment of third-stage larvae (L3). The first key process liable to disruption is the exsheathment of infective third stage larvae and the second is the ability of the exsheathed larvae to closely associate with the mucosal surface and enter into the digestive mucosae.

3) The question of the behavioral and physiological adaptations of goats to browse and to exploit TR plants and of the possible related consequences on the AH properties has currently received little attention but clearly deserves to be extensively explored.

Answers to these questions are necessary to make feasible the use of tanniniferous plant as a valuable option for the integrated control of gastrointestinal nematodes.

RESUMOS

EFFECTS OF CONDENSED TANNIN FROM *ACACIA MEARNsii* ON SHEEP INFECTED NATURALLY WITH GASTROINTESTINAL HELMINTHES

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The effect of tannins on endoparasite control in hair sheep was investigated using 20 entire lambs of the Santa Inês breed. At the beginning of the experiment these animals were 6-months old and weighed 22.5 kg \pm 4.7. The treatments used were (10 animals each): GT (animals receiving 18 g of Acácia negra containing 18% of condensed tannin/animal/week) and GC (animals not receiving tannin). The experiment lasted 84 days, with animals kept on an *Andropogon gayanus* pasture. Faeces were collected weekly, with weighing and blood collection carried out fortnightly. At slaughter, the adult worms were harvested for identification and counting. Although the GT animals weighed more than the GC lambs at slaughter, these differences were not significant ($P > 0.05$). In general, the values for haemoglobin, hematocrit, total protein, urea, phosphorus and calcium in the serum were within normal levels and no significant differences between groups were observed. For faecal egg count (FEC), lower values were observed throughout the experiment in the group receiving tannin, but these differences were only significant in the eighth week. There was a lower output of eggs by regression for GT compared with GC ($P < 0.05$). The species identified, in decreasing order of worm count, were: *Trichostrongylus colubriformis*, *Haemonchus contortus*, *Oesophagostomum columbianum*, *Cooperia* sp., *Strongyloides papillosus*, *Trichuris globulosa* and *Moniezia expansa*. The total worm count and number of each species of worm were lower for GT compared with GC for *T. colubriformis* and *Cooperia* sp. ($P < 0.05$). Condensed tannin (CT) from Acacia negra had an antiparasitic effect, thereby representing an alternative for worm control in sheep.

EFFECTS OF *AZADIRACHTA INDICA* ON SHEEP INFECTED NATURALLY WITH HELMINTHES

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The objective of this study was to evaluate the effects of neem (*Azadirachta indica*) on natural helminthes infection in lambs. Forty, four-month-old entire Santa Ines lambs, with average live weight of 11 ± 2.7 kg were grazed on an *Andropogon gayanus* pasture, over a 20 week period. They were divided into 4 treatments: without drenching (ND), 3g *A. indica*/animal (A₃), 6g *A. indica*/animal (A₆) and 9g *A. indica*/animal (A₉) over 5 consecutive days, with an interval of 28 days between drenching. Faeces were collected weekly and lamb weight and blood collection were carried out fortnightly. Four weeks after the last drenching all lambs were slaughtered, and worm burdens calculated. No significant differences were observed for lamb performance between treatments. Only treatment A₃ showed faecal egg counts (FEC) lower than treatment ND from the 12th week, although this was also not significant. Blood parameters highlighted the progress of the worm infections, with sheep on treatments A₆ and A₉ ending the experiment showing anemia. There was an increase in the total number of worms associated with increasing levels of neem. The increasing doses of neem did not improve the control of endoparasites in sheep naturally infected.

PERFORMANCE OF HAIR SHEEP AT PASTURE, SUPPLEMENTED WITH TROPICAL LEGUME (*MIMOSA CAESALPINIIFOLIA* BENTH) RICH IN CONDENSED TANNINS

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Sansão do campo (*Mimosa caesalpinifolia* Benth), a tropical legume with origin in the semi-arid region of Brazil containing approximately 12% condensed tannin (CT), was used to evaluate its effect on the performance of hair sheep at pasture. Eighteen lambs, with live weight of 16.9 ± 1.87 kg, were divided in three treatments: control (C) (68% corn + 32% soybean meal), sansão do campo (S) (60% sansão do campo + 30% corn + 10% soybean meal) and sansão do campo with polyethylene-glycol 4000 (S+PEG) (S + 10 g PEG/animal/day). All sheep were kept together on a 1 hectare of *Panicum maximum* Jacq. cv. Tanzânia pasture. Each individual animal received 200g/day of the supplement. CT content did not exceed 3% of dry matter (DM) in the diet. Pasture was collected and animals weighed fortnightly. Feed was analyzed for DM, crude protein (CP), Neutral-detergent fiber (NDF), Acid-detergent fiber (ADF) ether extract (EE) and ash (A). Concentrate analyses for group C (879, 214, 289, 49, 15 and 16 g/kg DM respectively) and S (876, 210, 532, 189, 24 and 15 g/kg DM respectively) as well as for forage (283, 89, 658, 388, 27 and 48 g/kg DM respectively) were available. After 105 days, group C lambs showed higher ($P < 0.05$) live weight (27.4 kg), total gain (10.1 kg) and mean daily gain (96.5 g/day) compared with group S (22.7 kg, 6.0 kg and 56.8 g/animal/day respectively) and group S+PEG (23.8 kg, 7.0 kg and 66.8 g/animal/day, respectively). Although animals from group S+PEG showed higher values than S for the traits analyzed, the differences were not significant ($P > 0.05$). The best performance in the control group was due to the higher available energy level in this supplement. Although the CT in the sansão do campo had little effect on the performance of the sheep, little is known about its effect over long periods of supplementation.

**Evaluation of the sandpaper extract (*Curatella americana* L)
(Dilleniaceae) on eggs and larvae of cyathostomin nematode
(Nematoda-Strongylidae)**

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Introduction

Several extracts have been tested *in vitro* and *in vivo* with the objective of controlling from an aggressive less way to the atmosphere, adults and larvae of several helminths (AMORIM et al., 1987; 1999; PACIORNIK, 1990; CUNHA et al., 2003, 2005; OLIVEIRA & AKISUE, 1997) A lot of plants accumulate organic substances that can be extracted in enough amount seeking the use in varied scientific applications (BALANDRIN et al. 1985).

The control through plants should promote the much slowest development of the resistance, it reaches only species objective, be biodegradable, pollute not the environment, and reduce the problem of environmental residues (WOUNDS, 2004). Phytochemical investigations resulted in the isolation of 3 composed through the mass spectrometry, characterized as ursólic acid, acetate of ursólic acid and betulínic acid (ALEXANDRE-MOREIRA, et al., 1999). Phytochemical studies with extract etanólico of leaves of *Curatella americana* revealed a glicosid flavonoid avicularim and galic acid (EL-AZIZI, 1980). The aim of this study was to evaluate the antthelminthic activity of extract of the plant Sandpaper (*Curatella americana*) through *in vitro* the egg hatching and larval development the on cyathostomin eggs and larvae eggs (Nematoda-Strongylidae).

Material and Methods

The aerial parts of *C. americana* were collected in Tres Marias, MG, Brasil, identified by botanists of the Botany Department of Federal Rural University and the a voucher specimens was deposited with numbers RBR 28008. In the Laboratory of Helminthologia of EPPWO Neitz of DPA -UFRRJ,RJ, feces samples were collected to OPG (eggs/g/feces) and coproculture (L3/g). Eggs were recovered through Rodrigues and Honer (1985) technique. A suspension of fresh eggs (300) cyathostomin with four replics, were submitted to action of aqueous extract of originating from *C.americana* Three Marias-MG in the concentrations of 0,5 and 1%, for 24, 48 and 72h.

Results

Activity of the aqueous extract of *C. americana* was not observed in the eggs hatching of the cyathostomin nematodes. The efficacy on the development larve L1 with the extract to 0,5%, was of 73,5% and for the concentration to 1% of 86,27%.

Conclusion

The aqueous extract of *C. americana* didn't present effect in in the eggs hatching. The extract presented high efficacy on the development first stage larvae. More studies in *vivo* must be performed to determine the effects of this extract.

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In vitro* effect of acacia extract on *Haemonchus contortus

infective larvae

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Introduction - Some *in vitro* methods can be used to investigate the efficacy of potential anthelmintic compounds towards nematode parasite. The assay, which analyses the larval migration inhibition (LMI), uses infective larvae in presence of potential anthelmintic substances. Athanasiadou et al. (2001) Related that LMI and larvae feed inhibition are the best assay to test anthelmintic effect of bioactive compounds *in vitro*.

Objective The main objective of this study is to determine the potential inhibitory effect of condensed tannins (CT) deriving of *Acacia mearnsii* extract (AE) on third stage larvae (L3) of *Haemonchus contortus*.

Material and methods - The eggs were obtained from faeces collected from donor lambs carrying monoespecific infections of *H. contortus*. Was used an ivermectin-resistant Brazilian strain isolated on IB – UNESP – Botucatu / SP. Coprocultures were carried out to isolate the L3, which were exsheathed and the solution adjusted so that 100ml contains approximately 100 larvae. Stock solution (200 mg mL⁻¹) was made by dissolving the AE in distilled water. Eight serial dilutions of tannin extract (200 mg mL⁻¹ to 35 mg mL⁻¹) were made deriving it. All concentrations of AE and the distilled water control were tested in triplicate. Firstly, approximately, 100 larvae were dispense into a series of labelled eppendorf tubes and were added of 1 ml of the tested AE concentration. After centrifugation the supernatant were removed and the larvae resuspended using 1 ml of the same solution. This action was repeated three times, after this the L3 were incubated in the appropriate AE concentration, for 2 hours at 37°C. A test plate (24 well) was added to 1800µl

AE solutions, including a filter in each used well. One sample of 200µl solution containing the larvae pre-incubated was added into each filter. After another 2-hour incubation at 37°C, the number of L3 that passed through the mesh and the restrained larvae in the filter were counted. To confirm the CT activity on L3 migration each assay were repeated in presence of PEG. The objective of using PEG is inactivating the most of CT effect proceeding from the AE. Ten micro liters of PEG solution were introduced in each eppendorf tube before the first L3 incubation.

Results - The percentages (mean of triplicate) of L3 that have migrated through the mesh (viable larvae) are shown with and without the presence of PEG (**Table 1**). The predicted value to reduce in 50 % the number of L3 that have migrated through the mesh (LD₅₀) was 63,8 mg.mL⁻¹ (R² = 0,98).

Table 1 Average (triplicate) from percentage (%) of third stage larvae of *Haemonchus contortus* that in presence of acacia extract (AE) migrates through the mesh. Results are shown with and without the presence of Polyethylene glycol (PEG).

| AE concentration mg.mL ⁻¹ | <i>Haemonchus contortus</i> (L3) | |
|---|----------------------------------|------------------------|
| | AE (migration %) | AE + PEG (migration %) |
| Control | 82,11 ^a | 82,0 ^a |
| 200 | 2,03 ^g | 24,85 ^c |
| 150 | 6,80 ^{fg} | 51,09 ^{bc} |
| 100 | 20,92 ^{ef} | 55,30 ^{bc} |
| 75 | 48,44 ^{cd} | 60,82 ^{ab} |
| 65 | 55,14 ^{bc} | 80,0 ^a |
| 50 | 66,38 ^{abc} | 82,0 ^a |
| 35 | 73,10 ^{ab} | 82,0 ^a |

Different letter in the same column - P <0.05

Conclusion - The results suggest the deleterious effect of AE on L3 larvae of *H. contortus* affecting their motility during *in vitro* assays.

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Effects of condensed tannins from *Acacia mearnsii* on *Trichostrongylus colubriformis* in experimentally infected sheep

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Introduction - The over-dependency and misuse of the chemotherapeutic drugs available to parasite control with consequent development of anthelmintic resistance, mainly in small ruminants, has been demanded alternative sources to control gastrointestinal nematodes in sheep.

Objectives - The aim of the present study was to investigate the potential anthelmintic effects of condensed tannins (CT) from acacia extract (AE) on *Trichostrongylus colubriformis* in experimentally infected sheep.

Materials and methods - Eighteen four-month-old worm-free Santa Inês lambs were divided in three groups and housed indoors-feeding hay and concentrate (diet with 13% of crude protein). Twelve animals were experimentally infected with 20,000 L3 of *T. colubriformis* and six were kept as non-infected control. Group I (GI) was the non-infected control; group II (GII) was infected control and group III (GIII) was supplied with condensed tannin (CT) source. The used source was the acacia extract (AE) from *Acacia mearnsii*, which contained 15% of CT. Thirty days post-infection (p.i.), Group III was supplemented with AE (1.6 g/kg of body weight) per day added to concentrate, during 10 days. The same amount (1.6 g/kg of B.W.) of dry molasses powder (DMP) was mixture with the AE to improve the diet palatability. Animals from Group II were supplemented only with DMP in the same dose of GIII. Faecal eggs counts (FEC) were carried out weekly until day 21p.i., twice a week from

day 21 to 30 p.i. and each two days during and after the treatment with CT (from day 30 to 45 p.i.). During the week of evaluation of AE, five coprocultures and egg viability tests were carried out for each infected group (days: 0, 4, 7, 10 and 14 of CT treatments) and the number of infective larvae (L3) per gram of faeces (LPG) and number of L1 reached from eggs (egg viability percentage) was determined. The values of FEC, LPG, egg viability, number of FEC accumulated per animal during the treatment period, number of viable eggs released, and worm burden were analyzed using the general linear model (GLM) with repeated measurements procedure of SAS. The means were compared by Duncan test and the differences between means with $P < 0.05$ were considered statistically different.

Results - On day 32 p.i. (second day of treatment with AE) a significant reduction on FEC ($P < 0.05$) was detected between GIII and GII. However, analyzing the average of total accumulated amount of FEC released per group, during the 10 days of the treatment, a significant reduction in CT treated lambs ($P < 0.05$) was detected, with FEC averages, per animal, during this period, of 11500 and 5317, respectively for GII and GIII. The values of egg viability, analyzed on days 0, 4, 7, 10 and 14 post-treatment (p.t.), were statistically reduced ($P < 0.05$) on days 4, 7 and 10 p.t. (Table 1). In the same way, values of viable eggs (FEC values x egg viability %) per group were lower in CT treated group ($P < 0.05$) on days 4 and 10, with tendency ($P = 0.06$) of reduction on day 7. The number of L3 recovered per gram of faeces (LPG) was lower ($P < 0.05$), at the days 10 and 14 p.t., in Group III (Table 1). The use of CT on diet caused no significant difference on body-weight and worm burden ($P > 0.05$). Animals from GI have not presented nematode eggs on faeces.

Table 1 Number of third stage larvae per gram of faeces (LPG) and egg viability (L1%) of *Trichostrongylus colubriformis*. In faeces samples collected from lambs from infected control groups (GII) and group treated with acacia extract during 10 days (GIII).

| G | Days Post-Treatment | | | | | | | | | |
|------|---------------------|-----------------|--------------------|-------------------|-------------------|-------------------|-------------------|-------------------|--------------------|--------------------|
| | 0 | | 4 | | 7 | | 10 | | 14 | |
| | LPG | L1 % | LPG | L1 % | LPG | L1 % | LPG | L1 % | LPG | L1 % |
| GII | 5790 ^a | 71 ^a | 15690 ^a | 90,5 ^a | 5340 ^a | 71,5 ^a | 1170 ^a | 31,5 ^a | 47400 ^a | 98,5 ^a |
| GIII | 11910 ^a | 70 ^a | 3900 ^a | 9,5 ^b | 1830 ^a | 54,5 ^b | 0 ^b | 3 ^b | 900 ^b | 59,50 ^a |

Different letter on the same column – $P < 0.05$

Conclusions Probably, the anthelmintic effect is not only originated by tannins and others secondary plants metabolites could be involved. However, the CT uses in nematode control cannot be discarded, not even their use, at least in alternative and prophylactic controls, reducing the pasture contamination, nematode reinfection in sheep and the number of chemical treatments.

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