Anthelmintic effects of condensed tannins on *Trichostrongylus colubriformis* in experimentally infected sheep

Efeito anti-helmíntico de taninos condensados sobre *Trichostrongylus colubriformis em* ovinos experimentalmente infectados

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Abstract

Recent surveys have identified anthelmintic effects from many bioactive substances particularly from condensed tannin (CT) sources. The aims of the present study were to investigate the potential anthelmintic effects of condensed tannins (CT) on *Trichostrongylus colubriformis* in experimentally infected sheep and the nutritional consequences on animals. Twenty helminth-free lambs were divided into five groups of four animals. Groups I to IV were artificially infected with 6,000 third stage larvae (L3) of *T. colubriformis*. Group I was the infected control and group V was the uninfected control. Twenty-eight days post-infection (p.i.) lambs from GII were supplemented with tanniniferous sorghum (350 g/animal/day, during seven days); GIII were drenched with *Acacia mearnsii* extract (15% CT) for just one day and GIV during two days (1.6 g extract/kg BW). At day 36 p.i., animals from infected group (GI to GIV) were slaughtered. Faecal egg counts (FEC) values present a reduction on GII when compared with GI at day 29 p.i. ($P < 0.05$) and between GIII and GI at day 35 and 36 p.i. ($P < 0.05$). The values of egg hatchability and number of L3 recovered from the faeces were not statistical analyzed (there was no duplicate data), however there was a considerable reduction between the values from treated and control group. The use of CT on diet did not cause significant difference on blood parameters, body-weight and carcass-weight ($P > 0.05$). No difference was related on total worm burden between treatments; however, GIV presented lower number of females than GI ($P < 0.05$). The use of CT could be a promising alternative source to reduce the pasture contamination and to control *T. colubriformis* infection in sheep.
Key words: Nematode, ovine, alternative control, bioactive compounds, tannins

Resumo

O objetivo principal deste trabalho foi avaliar o potencial efeito anti-helmíntico dos taninos condensados sobre *Trichostrongylus colubriformis* em ovinos experimentalmente infectados e as conseqüências nutricionais destes produtos sobre os animais. Vinte ovinos livres de infecção parasitária foram divididos em cinco grupos de quatro animais. Os grupos GI a GIV foram experimentalmente infectados com 6000 larvas infectantes (L3) de *T. colubriformis.* O GI foi mantido como controle infectado e o GV como controle não infectado. Vinte e oito dias pós-infecção (p.i.) os animais foram tratados com fontes de taninos condensados (TC): o GII suplementado com 350 g de sorgo taninífero, durante sete dias; os

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grupos GIII e GIV receberam extrato de *Acacia mearnsii* (1,6 g de extrato/kg de peso vivo), durante um ou dois dias, respectivamente. No 36º dia p.i. os animais dos grupos I a IV foram abatidos. No dia 29 p.i. (2º dia de tratamento) foi detectada diferença significativa no número de ovos por grama de fezes ($P < 0,05$) entre o GII e GI; já no 35º e 36º dias p.i. houve diferença ($P < 0,05$) entre os grupos GIII e GI, com menor número de ovos no grupo tratado com TC. A carga parasitária total dos grupos foi semelhante, porém houve diferença significativa no número de fêmeas do GIV em relação ao GI ($P < 0,05$). Não houve alteração no peso corporal e nos parâmetros hematimétricos dos animais tratados com fontes de TC ($P > 0,05$). O uso dos TC pode ser promissor no controle de *T. colubriformis* em ovinos.
Palavras-chave: Nematóides, ovinos, controle alternativo, compostos bioativos, taninos

Introduction

The anthelmintic drugs promote significantly decrease on the worm burden in lambs, however, the development of anthelmintic resistance in worm populations is now a worldwide phenomenon, in constant expansion (JACKSON; COOP, 2000), mainly in small ruminants. Many factors are involved on the development of drug resistance as: genetic basis and number of involved genes; frequency, dose and efficacy of anthelmintic treatment (SARGISON et al., 2006).

Many plants have been used in parasites control programs worldwide and despite ample evidence of anthelmintic properties of them, little research had been done to validate their use, especially in veterinary medicine (GITHIORI; ATHANASIADOU; THAMSBORG, 2006).

Assays using sheep fed condensed tannins (CT) deriving from forages or plants extracts (Acacia and Quebracho) have been supported to develop alternative controls to gastrointestinal parasites, among them *T. colubriformis* (ATHANASIADOU et al., 2000a; 2000b). Some authors have reported a relatively good effect of CT on worm burden of intestinal (ATHANASIADOU et al., 2001b) and abomasum worms (HÖRDEGEN et al., 2003; MIN; HARTT, 2003) after the use of CT in ruminant diet.

The main objective of this work was to evaluate the effect of CT from acacia extract (AE) and from tanniniferous sorghum (*Sorghum bicolor*) on sheep experimentally infected with *T. colubriformis*. With this purpose were evaluated: (1) the number of helminths eggs per gram of faeces (FEC); (2) the worm burden and (3) the effects on animal production, according to the variables body weight (BW), carcass weight and voluntary food intake.

Material and Methods

Experimental design

The experimental lambs were housed indoors feeding hay and concentrate. Sixteen animals were experimentally infected with 6,000 L3 of *T. colubriformis* and four were kept as non-infected control. One day before the beginning of the treatments (28 days after infection), the animals were divided in four groups according their BW and FEC. Group I (GI) was the infected control; group II (GII) was supplied with tanniniferous sorghum, group III (GIII) was drenched once with acacia extract (AE); group IV (GIV) was drenched twice with AE and group V (GV) was the non-infected control.

During the week of evaluation of CT sources, coprocultures and egg hatchability tests were carried out for each experimental group at days: 0, 2, 5 and 7 of CT treatments. Two days after the beginning of the treatments, two lambs from each group were kept in metabolic cages to evaluate the voluntary food intake. Refusals of concentrate, hay and total amount of faeces were recorded. All infected animals (GI to GIV) were slaughtered nine days after the initial treatments with CT (at day 36 p.i.) for worm burden determination.

Condensed tannins sources

The sources of CT analyzed were: tanniniferous sorghum (S*orghum bicolor*) grain and acacia extract (*Acacia mearnsii*), which contained, respectively, 2% and 15% of CT according HCl-Butanol method (PORTER; HRSTICH; CHAN, 1986).

Animals and infective larvae

Twenty 3-month-old worm-free Santa Inês lambs were used in the experiment. *T. colubriformis* infective larvae (L3) were isolated from sheep donors with monoespecific infection. The L3 were kept in the refrigerator (5 ºC to 7 ºC) and used before had completed 21 days of storage.

Feeding and CT treatments

Animals were housed indoors feeding with the experimental diet composed by concentrate (maize + soybean meal) and hay (*Cynodum* sp.), with 13% of crude protein (CP) and supplemented *ad libitum* with a mineral mixture. The amount of concentrate and hay intake were calculated according to the NRC (1985), for BW, age and expected BW gain of 200 g/day/animal. The concentrate was offered twice a day and the quantity was adjusted weekly.

The group treated with tanniniferous sorghum grains was supplemented with 350 g per day added to concentrate during seven days (7 g of CT/animal/day). The acacia extract (AE) was diluted in 50 mL of water and drenched to animals once (GIII) or twice (GIV) on consecutive days on dose of 1.6 g/kg BW (7.2 g of CT per day for an animal with 30 kg of BW).

Body weight and blood parameters

The lambs were weighted once a week and blood samples were collected each 15 days with EDTA for globular volume and hemoglobin determinations (microhematocrit and colorimetric methods, respectively).

Parasitological techniques

Faecal eggs counts (FEC) were carried out weekly until day 21p.i., twice a week from day 21 to 27 p.i. and daily during the treatment with CT (from day 28 to 36 p.i.). The egg counts were performed according to a modified Mc Master technique (UENO; GONÇALVEZ, 1994).

Slaughter and worm count procedures

Two days after the end of the treatments (36 days after-infection), animals from infected groups (GI to GIV) were slaughtered and the parasitological necropsy was carried out according Wood et al. (1995). The abomasum was removed, tied and opened to recover its content, and later the intestinal mucosa was washed and scraped with a clipper handle to grasp all worms. For worm burden determination a 10% volume aliquot from total gut content was taken in duplicate and fixed with formalin 10%. For total worm burden determination one 10% aliquot was used.

Statistical analysis

The FEC values were analyzed after logarithmical transformation [log (x+10)]. The values of FEC, body weight, mean globular volume and worm burden were analyzed using the general linear model with repeated measurements procedure of SAS (1996). The averages were compared using the Tukey test (OTT, 1992) and the differences at the 5% level were considered significant.

Results

Body weight

On day 21 p.i., when the experimental groups were divided, the BW (kg) average per group was: GI 15.65 kg, GII 15.45 kg, GIII 15.48 kg, GIV 15.45 kg and GV 15.25 kg (SE: 0.581; $P > 0.05$).

Significant difference of BW among the treatments during the experimental period was not observed (P > 0.05) and the average of BW at slaughter, 36 days p.i. was: GI 19.60 kg, GII 19.30 kg, GIII 19.95 kg, GIV 20.05 kg and GV 21.15 kg (SE: 1.401; P > 0.05).

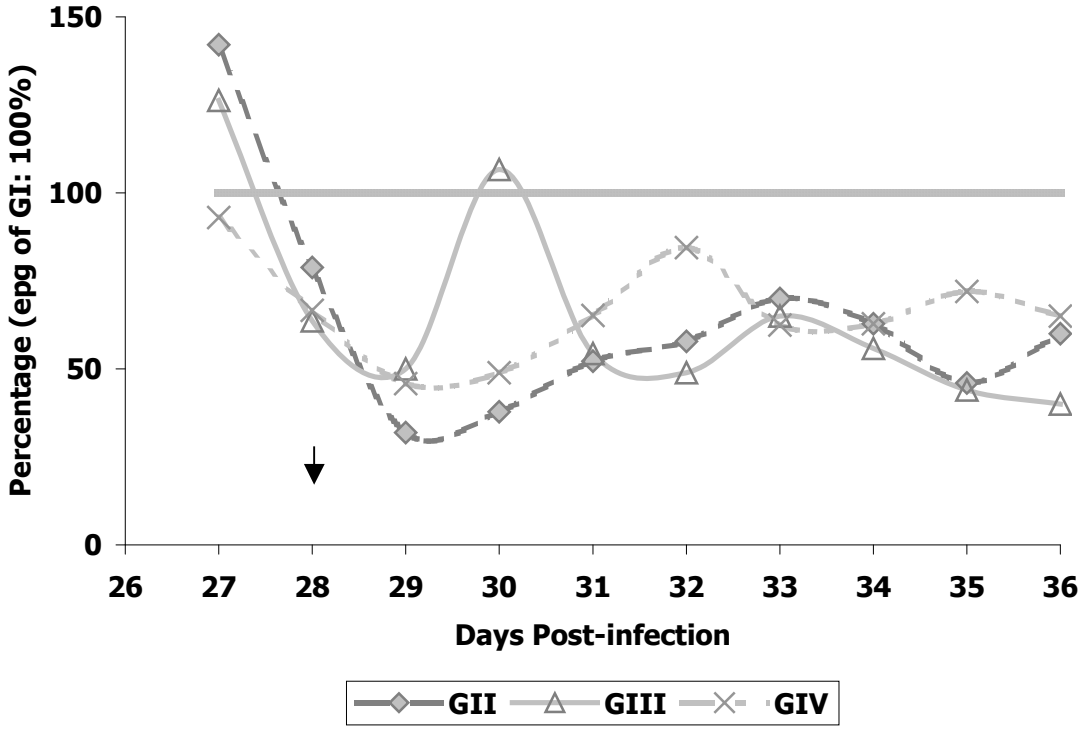
Packed cell volume and hemoglobin

The PCV and hemoglobin mean values did not present statistical differences during the assay (P > 0.05). The average of MGV (%) values per groups on the last week of the experiment were: GI 34.5, GII 37.5, GIII 35.5, GIV 36.0 and GV 36.25 (SE: 1.59; P > 0.05), and the hemoglobin values (g/dL) were: GI 12.09, GII 13.26, GIII 11.70, GIV 11.11 and GV 12.28 (SE: 0.83; P > 0.05).

Faecal egg counts (FEC)

On day 29 p.i. (second day of treatment with CT) a significant reduction on FEC (P < 0.05) was detected only between the treatments GI (infected control) that presented a mean value of 975 eggs per gram of faeces (epg) and GII (sorghum) with a value of 725 epg. There was also a significant difference (P < 0.05) on days 35 and 36 p.i. between GIII and GI with GIII presenting 750 and 575 epg and GI, 1075 and 975 epg at days 35 and 36 respectively.

Animals of GV (non-infected control) remain negative during all experimental period. Figure I presented FEC reduction (%), per group compared to GI (non treated infected control), during the experimental period.



↓: Arrow indicates the first day of treatment with CT

Figure 1. Variation in eggs per gram of faeces (epg) released by lambs experimentally infected with *Trichostrongylus colubriformis* and divided in four groups: treated with sorghum during seven days (GII), treated once (GIII) or twice-consecutive days (GIV) with acacia extract, compared to (GI) infected control non-treated. Values are presented in percentage and GI is demonstrated by the straight line (standard 100%).

Worm burden

The total worm burden values per group had no significant differences; however, GIV presented lower number of females than GI (P < 0.05). On Table I, the mean values of worm burden and mean number of *T. colubriformis* male and female (minimum and maximum) were presented.

Table 1. Mean worm burden (male, female and total) in lambs experimentally infected with 6000 L3 of *T. colubriformis* and treated with sorghum (GII), treated once (GIII)) and twice (GIV) with acacia extract and infected no-treated control (GI), slaughtered 36- days post-infection.

Groups	Number of *T. colubriformis* (minimum; maximum)		
	Male	Female	Total
GI	**667.5**[a] (510; 930)	**1335**[a] (110; 1710)	**2002,5**[a] (1540; 2640)
GII	**825**[a] (400; 1600)	**1132.5**[ab] (860; 1700)	**1957.5**[a] (1260; 3300)
GIII	**522.5**[a] (460; 650)	**1002.5** [ab] (910; 1060)	**1525**[a] (1380; 1710)
GIV	**490**[a] (460; 530)	**850**[b] (610; 1320)	**1340**[a] (1070; 1830)

Mean followed by different letters in the column are significantly different (P < 0.05).

Voluntary intake

The CT supply, either as sorghum grains or as AE, had no effect on voluntary food intake, refusals of concentrated and hay and the total amount of faeces (mean) of the sheep from the different treatments were similar (P > 0.05).

Discussion

The mean FEC presented significant reductions in animals treated with sorghum (GII) and with AE (GIII) when compared with infected control group (GI). Initially, animals from GII presented lower reduction, followed by GIII on the end of treatment period, confirming the potential use of taniniferous plants in the nematodes control. Many experiments correlated the CT uses with decrease of female helminthes fecundity (ATHANASIADOU et al., 2000a, 2001a; MIN; HART, 2003; PAOLINI et al., 2003a, 2003b), standing out its importance in nematode epidemiology

Despite the lower epg values from animals treated with CT in this study, the number of total worms presented in the intestines of sheep from different treatments was similar (P > 0.05). However, sheep treated twice with AE, which did not present the lower epg, showed a significantly lower number of nematode female.

It was observed a higher variation related to worm burden and epg, among sheep from the same treatments. This fact, associated with the relatively short number of animals per group, could influence the results, but it was clear the tendency that lower values been presented in the treated groups.

Minho et al. (2008a), working with lambs naturally infected with *H. contortus* and *T. colubriformis*, related that the AE drenching two consecutive days per month presented a significant reduction in the FEC and worm burden in abomasum, but not in small intestine. Cenci et al. (2007), working with the same AE source (once a week for eight weeks), in naturally infected sheep, also related significant decrease on FEC. I*n vitro* studies with AE found inhibitory effect of this compounds on the feeding capability of first stage larvae of *H. contortus, Trichostrongylus vitrinus* and *Teladorsagia circumcincta* with LD_{50} 0.043, 0.038 and 0.050 mg of AE/ml, respectively (MINHO et al., 2008b).

However, Athanasiadou et al. (2000b) reported that the epg values reduction in treated animals could not be attributed only to CT. Butter et al. (2000) demonstrated reduction on faecal egg counts (FEC) in animals treated with CT sources, but the FEC were similar to that observed for sheep from the group that had received only high protein diet.

In this study, the amount of CT from sorghum or AE cause no significant gastrointestinal disturbs in sheep or losses on feed intake. Pasty diarrhea were observed in three animals (two from group GIII and one from group GIV), during two days after treatment with CT. Minho et al. (2008) and Cenci et al. (2007) also with the same dose of AE used in this assay, in sheep naturally infected, did not observed gastrointestinal disturbs.

Parasitic infection usually reduces voluntary intake from 10 up to 30% (COOP AND SYKES, 2002), even in sub clinical infections (KYRIAZAKIS et al., 1996). However, in the current study, probably due the short experimental period, significant difference on food intake was not detected ($P > 0.05$) among the different treatments. Athanasiadou et al. (2001a), evaluating the use of quebracho extract (60 g/kg DM), during 67-day period, to control *T. colubriformis* infection in sheep observed no increase on the BW, however, in the same study, the association of CT and high protein diet increased the food intake and controlled the nematode infection without damage on digestibility.

Significant difference was not observed on mean BW values, with non-infected control group presenting the highest BW mean; however, no statistical difference was detected. The number of *T. colubriformis* L3 recommended for a simple infection, in sheep, can vary from 3,000 to 6,000 (WOOD et al., 1995). However, the infective dose of 6,000 L3, used in this work, cause no clinical sighs and no effect on BW, probably due the short time of observation or due the greater capacity of Santa Inês lambs to stand the adverse effects of nematode infection (ROCHA; AMARANTE; BRICARELLO, 2005) and also some effect of patogenicity of the *T. colubriformis* strain used in the assay.

The aim of this study was to confirm the potential use of CT as supplementation in ruminant diets, as prophylactic in parasite controls, as recommended by many authors (ATHANASIADOU et al., 2001a; NIEZEN et al., 2002a; PAOLINI et al., 2003a; HOSTE; GAILLARD; FRILEUX, 2005; MINHO et al., 2008a). Nevertheless, to correlate the CT consumption with a reduction of nematode eggs on pasture. Both direct and indirect effects of CT are beneficial in lowering the contamination of pasture by reducing the hatchability of nematode eggs and faecal egg count reduction (IQBAL et al., 2007).

Worldwide the search for an ideal CT source to nematode control in small ruminants is farm to finish. In this study, the choice of the extract from AE was influenced by the fact of its concentration of CT present little variation during the year and it is easily available commercially. Moreover, quebracho extract (QE) from *Schinopsis brasilienses* tree is in extinguishing process in Brazil.

Ketzis et al. (2006) suggest that parasitologists, epidemiologists, clinicians, nutritionists, economists, and livestock producers may all have different views about an alternative control program; therefore their opinion would have to be considered. By consequence, other studies are necessary to evaluate: the direct anthelmintic effect of CT sources on *T. colubriformis*, mainly CT extracts, and its effect on the different helminth stages on environment, before having its widespread use as anthelmintic to small ruminants.

Conclusions

The AE drenched twice-consecutive days reduce the number of nematode female in treated animals. The treatments with taniniferous sorghum and AE once were effective in reduce FEC.

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Ethical approval

This experiment was approved by the Animal Experimentation Ethics Committee of the Nuclear Energy Center for Agriculture (CENA), São Paulo University (USP), Brazil.

References

ATHANASIADOU, S.; KYRIAZAKIS, I.; JACKSON, F.; COOP, R. L. Consequences of long-term feeding with condensed tannins on sheep parasitised with Trichostrongylus colubriformis. *International Journal for Parasitology*, Oxford, v. 30, n. 9, p. 1025-1033, 2000a.

_____. Direct anthelmintic of condensed tannins towards different gastrointestinal nematodes of sheep: in vitro and in vivo studies. *Veterinary Parasitology*, Amsterdam, v. 99, n. 3, p. 205-219, 2001b.

_____. Effects of short-term exposure to condensed tannins on adult Trichostrongylus colubriformis. *Veterinary Record*, London, v. 146, n. 25, p. 728-732, 2000b.

_____. The effects of condensed tannins supplementation of foods with different protein content on parasitism, food intake and performance of sheep infected with *Trichostrongylus colubriformis*. *British Journal of Nutrition*, Cambridge, v. 86, n. 6, p. 697-706, 2001a.

BUTTER, N. L.; DAWSON, J. M.; WAKELIN, D.; BUTTERY, P. J. Effect of dietary tannin and protein concentration on nematode infection (*Trichostrongylus colubriformis*) in lambs. *Journal of Agricultural Science*, Cambridge, v. 134, n. 1, p. 89-99, 2000**.**

CENCI, F. B.; LOUVANDINI, H.; DELL`PORTO, A.; MCMANUS, C.; COSTA, D. M.; ARAÚJO, S. C. de; MINHO, A. P.; ABDALLA, A. L. Effects of condensed tannin from *Acácia mearnsii* on sheep infected naturally with gastrointestinal helminthes. *Veterinary Parasitology*, Amsterdam, v. 144, p. 132-137, 2007.

COOP, R. L.; SYKES, A. R. Interactions between gastrointestinal parasites and nutrients. In: FREER, M.; DOVE, H. (Ed.). *Sheep nutrition*. Wallingford: CABI, 2002. p. 313-331.

GITHIORI, J. B.; ATHANASIADOU, S.; THAMSBORG, S. M. Use of plants in novel approaches for control of gastrointestinal helminths in livestock with emphasis on small ruminants *Veterinary Parasitology*, Amsterdam, v. 139, n. 4, p. 308-320, 2006.

HÖRDEGEN, P.; HERTZBERG, H.; HEIMANN, J.; LANGHANS, W.; MAURER, V. The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. *Veterinary Parasitology*, Amsterdam, v. 117, n. 1, p. 51-60, 2003.

HOSTE, H.; GAILLARD, L.; FRILEUX, Y. L. E. Consequences of the regular distribution of sainfoin hay on gastrointestinal parasitism with nematodes and milk production in dairy goats. *Small Ruminant Research*, v. 59, n. 2/3, p. 265-271, 2005.

IQBAL, Z.; SARWAR, M.; JABBAR, A.; AHMED, S.; NISA, M.; SAJID, M. S.; KHAN, M. N.; MUFTI, K. A.; YASEEN, M. Direct and indirect anthelmintic effects of condensed tannins in sheep. *Veterinary Parasitology*, Amsterdam, v. 144, n. 1/2, p. 125-131, 2007.

JACKSON, F.; COOP, R. L. The development of anthelmintic resistance in sheep nematodes. *Parasitology*, Cambridge, v. 120, p. 95-107, 2000.

KETZIS, J. K.; VERCRUYSSE, J.; STROMBERG, V. E.; LARSEN, M.; ATHANASIADOU, S.; HOUDJIK, J. G. M. Evaluation of efficacy expectations for novel and non-chemical helminth control strategies in ruminants. *Veterinary Parasitology*, Amsterdam, v. 39, n. 1, p. 321-335, 2006.

KYRIAZAKIS, I.; ANDERSON, D. H.; OLDHAM, J. D.; COOP, R. L.; JACKSON, F. Long-term subclinical infection with Trichostrongylus colubriformis: effects on food intake, diet selection and performance of growing lambs. *Veterinary Parasitology*, Amsterdam, v. 61, n. 3/4, p. 297-313, 1996.

MIN, B. R.; HART, S. P. Tannins for suppression of internal parasites. *Journal of Animal Science*, Champaign, v. 81, n. 2, p. 102-109, 2003.

MINHO, A. P.; BUENO, I. C. S.; GENNARI, S. M.; JACKSON, F.; ABDALLA, A. L. I*n vitro* effect of condensed tannin extract from acacia (*Acacia mearnsii*) on gastrointestinal nematodes of sheep. *Revista Brasileira de Parasitologia Veterinaria*, Jaboticabal, v. 17, n. 1, p. 147-151, 2008b.

MINHO, A. P.; BUENO, I. C. S.; LOUVANDINI, H.; JACKSON, F.; GENNARI, S. M.; ABDALLA, A. L. Effect of acacia tannin extract in the control gastrointestinal parasites in sheep. *Animal Feed Science and Technology*, Riverport Lane, v. 147, n. 1/3, p. 172-181. 2008a.

NIEZEN, J. H.; WAGHORN, G. C.; GRAHAM, T.; CARTER, J. L.; LEATHWICK, D. M. The effect of diet fed to lamb on subsequent development of *Trichostrongylus colubriformis* larvae in vitro and on pasture. *Veterinary Parasitology*, Amsterdam, v.105, n. 4, p. 269-283, 2002.

NRC. National Research Council. Nutrient requirements of sheep. 6. ed. Washington: NRC, 1985. 138 p.

OTT, R. L. An introduction to statistical methods and data analysis. 4. ed. Belmont: Wadsworth Publishing Company, 1992. 1051 p.

PAOLINI, V.; BERGEAUD, J. P.; GRISEZ, C.; PREVOT, F.; DORCHIES, P. H.; HOSTE, H. Effects of condensed tannins on goats experimentally infect with *Haemonchus contortus*. *Veterinary Parasitology*, Amsterdam, v. 113, n. 3/4, p. 253-261, 2003b.

PAOLINI, V.; FRAYSSINES, A.; DE LA FARGE, S.; DORCHIES, P.; HOSTE, H. Effects of condensed tannins on established populations and in incoming larvae of *Trichostrongylus colubriformis* and *Teladorsagia* circumcincta in goats. *Veterinary Research*, v. 34, n. 3, p. 331-339, 2003a.

PORTER, L. J.; HRSTICH, L. N.; CHAN, B. G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry*, v. 25, n. 1, p. 223-230, 1986.

ROCHA, R. A.; AMARANTE, F. T.; BRICARELLO, P. A. Resistance of Santa Inês and Ile de France suckling lambs to gastrointestinal menato de infections. *Revista Brasileira de Parasitologia Veterinária*, Rio de Janeiro, v. 14, n. 1, p. 17-20, 2005.

SARGISON, N. D.; JACKSON, F.; BARTLEY, D. J.; WILSON, D. J.; STENHOUSE, L. J.; PENNY, C. D. Observation on the emergence of multiple anthelmintic resistance in sheep flocks in the south-east of Scotland. *Veterinary Parasitology*, Amsterdam, v. 145, n. 1/2, p. 65-76, 2007.

SAS INSTITUTE. *SAS User's guide*: statistics. Version 6.12. Cary, USA: North Caroline State University, 1996. 956 p.

UENO, H.; GONÇALVES, P. C. *Manual para diagnóstico das helmintoses de ruminantes*. 3. ed. Tokyo: Japan International Cooperation Agency, 1994. 166 p.

WOOD, I. B.; AMARAL. N. K.; BAIRDEN, K.; DUNCAN, J. L.; KASSAI, T.; MALONE JUNIOR, J. B.; PANKAVICH, J. A.; VERCRUYSSE, J. 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). *Veterinary Parasitology*, Amsterdam, v. 58, n. 3, p. 181-213, 1995.