

Effect of sericea lespedeza (*Lespedeza cuneata*) fed as hay, on natural and experimental *Haemonchus contortus* infections in lambs

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Abstract

Parasitic infection is one of the leading economic constraints in small ruminant production. This problem is exacerbated as the resistance of nematode populations to chemical treatment (anthelmintics) becomes increasingly more common. Condensed tannin (CT) containing plants are being investigated as alternative solutions to address these problems. This investigation was conducted to evaluate the effect of the CT containing forage, *Lespedeza cuneata* (sericea lespedeza, SL) fed as hay, on *Haemonchus contortus* infection in sheep. Naturally and experimentally infected lambs were fed either SL or bermudagrass (BG, control) hay for 49 days. All lambs were fed BG hay for an additional 14 days. SL hay effectively reduced (67–98%) fecal egg count (FEC) during the time of feeding for both infection groups. FEC increased in both infection groups after SL feeding was stopped which indicated an effect on fecundity. SL hay feeding also reduced worm numbers, with more of an effect on reducing naturally infected worm burdens (67.2%) than on establishment of incoming larvae (26.1%). SL fed as hay may be more useful to remove existing worms than establishing worms. The decrease in FEC would have the benefit of reduced pasture contamination.

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1. Introduction

Haemonchus contortus is a parasitic nematode that infects sheep, goats, deer, and other small ruminants and has been a significant cause of economic loss to

farmers of small ruminants worldwide, including the southeastern US. With overuse, most synthetic anthelmintics are becoming increasingly ineffective due to parasite resistance worldwide (Waller, 1994; Sangster, 1999) and in the U.S. (Miller and Barras, 1994; Zajac and Gipson, 2000; Terrill et al., 2001; Mortensen et al., 2003). There is also a growing concern over chemical residues in animal products and on pastures, and a worldwide increased demand for organic agricultural products for which use of

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synthetic chemicals is minimal (McKellar, 1997; Hördegen et al., 2003).

These factors all contribute to an increasing effort to find alternative and novel approaches to parasite control. Condensed tannin (CT) containing plants have gained interest as a promising alternative to traditional anthelmintics, and several studies have been conducted to ascertain their effectiveness *in vivo* (Niezen et al., 1995, 1998; Athanasiadou et al., 2000, 2001; Min and Hart, 2003; Paolini et al., 2003a,b,c; Shaik et al., 2004) and *in vitro* (Athanasiadou et al., 2001; Molan et al., 2002; Bahuaud et al., 2006).

In sheep, use of a CT extract (quebracho) showed an ability to decrease fecal egg count (FEC) with a decrease in the number of the small intestinal nematodes *Trichostrongylus colubriformis* and *Nematodirus battus*, but no effect on the abomasal nematodes *H. contortus* and *Teladorsagia circumcincta* (Athanasiadou et al., 2001). Paolini et al. (2003a) used quebracho extract on goats infected with *H. contortus*, and observed a significant decrease in FEC, but no difference in nematode numbers upon necropsy. Further studies by Paolini et al. (2003c) showed that the effect of CT on *T. colubriformis* and *T. circumcincta* infection in goats varied depending on the parasitic stage exposed (established versus developing infection).

CT in some fresh forages (sulla: *Hedysarum coronarium* L. and big trefoil: *Lotus pedunculatus*) has been shown to decrease FEC and worm counts, as well as to increase performance of parasitized sheep (Niezen et al., 1995, 1998). Hay of the CT containing forage sainfoin has also shown potential in controlling infection in goats (Paolini et al., 2003b, 2005a). Another forage plant that contains high levels of CT is *Lespedeza cuneata* (sericea lespedeza, SL, or Chinese bush clover). Grazing SL, as fresh forage, has been shown to reduce FEC and inhibit larvae development of *H. contortus* (Min et al., 2004), and SL fed as hay significantly reduced the FEC of goats that had natural and experimental infections of *H. contortus* (Shaik et al., 2004). In a follow-up study, Shaik et al. (2006) reported a direct inhibitory effect of SL hay on larvae in feces and adult worms in the abomasum and small intestine of goats. The objective of this investigation was to test the efficacy of SL fed as hay on reducing and/or preventing *H. contortus* infection in sheep.

2. Materials and methods

2.1. Study protocol

Two studies were conducted. Study 1 simulated a grazing situation to evaluate the effect of SL hay on

established natural infection while continuing to be reinfected during grazing. Study 2 simulated a grazing situation to evaluate the effect of SL hay on reinfection after existing natural infection was removed by anthelmintic treatment. Twenty-four naturally infected 4-month-old ewe lambs were removed from pasture and maintained in cement floored pens at the Louisiana State University, School of Veterinary Medicine in Baton Rouge, LA. For each study, 12 lambs were randomly allocated, based on FEC and weight, into 1 of 2 groups (6 animals each). For Study 1, lambs retained natural infections and for Study 2, lambs were dewormed with levamisole (Tramisol, 8 mg/kg) and albendazole (Valbazen, 10 mg/kg) on 2 consecutive days. Each pen housed two animals each. For 14 days, all animals were fed bermudagrass (BG: *Cynodon dactylon*) hay and then one group in each study was switched to SL hay (Day 0), and the remaining groups continued to be fed BG hay (control). On Day 49, the SL group in each study was switched back to BG hay. All hay was offered in excess in hay baskets placed in each pen. In addition, a 16% CP lamb finishing ration (Purina[®] Lamb Show Ration, Purina Mills, St. Louis, MO) was fed as a supplement at 0.23 kg per animal per day and water was available at all times.

On Day 0, all animals began receiving trickle infections (to simulate reinfection) of a mixed larval inoculum containing 1000 L3 (97% *H. contortus* and 3% *T. colubriformis*) three times a week for 3 weeks. L3 larvae were delivered in water solution orally using a 10 ml syringe. Feces and blood were collected weekly for determination of fecal egg count (FEC reported as eggs per gram, epg) and packed cell volume (PCV reported as percent). Feces were collected directly from the rectum and processed using a modified McMaster technique (Whitlock, 1948). Blood was collected by jugular venipuncture using 7 ml EDTA vacutainer tubes and PCV was determined using hematocrit tubes spun in a microhematocrit centrifuge.

On Days 21, 28 and 35, one animal in the control group of Study 1 became progressively anemic (PCV < 19) and was dewormed with levamisole (Tramisol, 8 mg/kg) and albendazole (Valbazen, 10 mg/kg). Data for this animal was discarded post-deworming.

At the end of each study period (Day 63), three lambs from each group were euthanized and necropsied. The three lambs were the ones with the highest, middle and lowest FEC. The abomasum, small intestine and large intestine were collected for nematode enumeration and identification in accordance with established procedures (Miller et al., 1987). The recovery procedure

was modified in that organ contents were brought to a volume of 5 l and a 500 ml aliquot was taken and preserved with formalin (10%).

2.2. *Sericea lespedeza* condensed tannin analysis

Samples of SL hay were ground and analyzed for CT content (Fort Valley State University, Fort Valley, GA). Extractable, protein-bound, and fiber-bound CT content was determined by the Terrill et al. (1992) method using purified quebracho CT as the standard (Kahiya et al., 2003). CT content was 3.6% extractable, 13.4% protein-bound and 5.4% fiber-bound for a total of 22.4% (quebracho equivalents).

2.3. Statistical analysis

2.3.1. FEC and PCV

The SAS statistical package (version 9.1.3) was used to analyze FEC and PCV as a repeated measures design in a split-plot arrangement of treatments with treatment group and animal within treatment group effects on the main plot and time and treatment group by time interaction effects on the subplot. FEC was natural log-transformed to stabilize variance (Winer, 1971). Pairwise comparisons of significant main effect comparisons were analyzed with Tukey's HSD test. Significant interaction effects were examined with pairwise comparisons of least-square (adjusted) means. Differences were considered significant when $p \leq 0.05$.

2.3.2. Worm counts

To stabilize variance terms, the data were natural log-transformed and *t*-tests were conducted. Due to the concerns for normality with small samples, nonparametric tests (Wilcoxon rank-sum) were generated. Differences were considered significant when $p \leq 0.05$.

3. Results

3.1. Study 1

3.1.1. Fecal egg count

Mean FEC was similar ($p > 0.05$) between BG (2692 epg) and SL (2450 epg) groups on Day 0 (Fig. 1). On Day 7, FEC of the SL group decreased compared to the BG group and remained significantly ($p < 0.01$) lower throughout the study. After SL feeding stopped (Day 49), the SL group FEC increased but remained lower than the BG group. Percent reduction in the SL group FEC, relative to the BG group, was 98% on Day 7

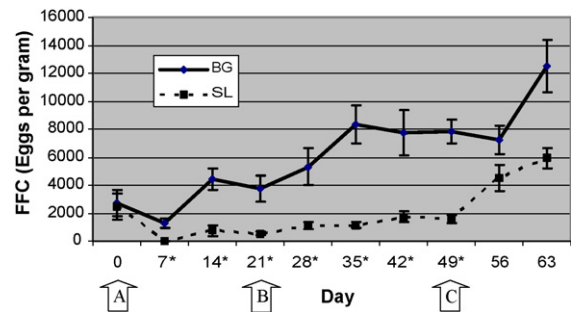


Fig. 1. Study 1: fecal egg count (FEC) for sericea lespedeza (SL) hay fed ($n = 6$) and bermudagrass (BG) hay fed ($n = 6$) lambs with natural and experimental *Haemonchus contortus* infection. (A) Hay feeding and experimental infections (1000 L3 3 times a week for 3 weeks) started. (B) Experimental infections stopped. (C) SL hay feeding switched to BG hay feeding. Asterisk (*) indicates significant ($n < 0.05$) difference based on log-transformed data.

and varied from 77% to 86% through Day 49 and dropped to 37% and 53% after SL feeding stopped.

3.1.2. Blood packed cell volume

Mean PCV was similar ($p > 0.05$) between BG (37.0%) and SL (35.5%) groups on Day 0. For both groups, PCV decreased continually through Day 35 and then stabilized at around 23% and 27% for BG and SL groups, respectively. The SL group remained significantly ($p < 0.01$) higher than the BG group for the entire study.

3.1.3. Worm count

The only worms found were *H. contortus* from the abomasum. None were found in the small or large intestine. Mean *H. contortus* count in the SL group (650) was lower than the BG group (1983) but the difference was not significant ($p > 0.05$). The female to male ratio was higher for the SL group (1.69) than the BG group (1.16), but the difference was not significant ($p > 0.05$).

3.2. Study 2

3.2.1. Fecal egg count

Mean FEC was similar (< 200 epg, $p > 0.05$) through Day 21 (Fig. 2). FEC in both groups increased starting Day 28, however, the SL group remained significantly ($p = 0.01$) lower than the BG group for the rest of the study. After SL feeding stopped (Day 49), the difference in FEC between groups was reduced, but the SL group remained lower than the BG group. Percent reduction in the SL group FEC, compared to the BG group, from Day 28 to Day 49 varied from 67% to 82% and dropped to 31% and 35% after SL feeding stopped.

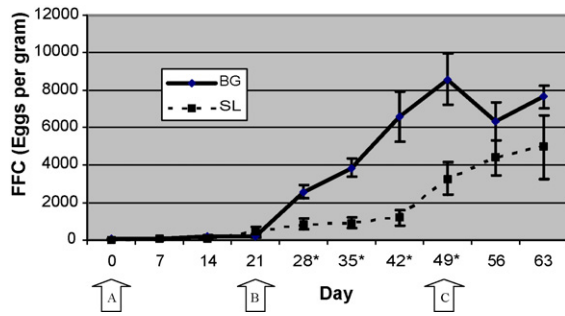


Fig. 2. Study 2: fecal egg count (FEC) for sericea lespedeza (SL) hay fed ($n = 6$) and bermudagrass (BG) hay fed ($n = 6$) lambs with experimental *H. contortus* infection. (A) Hay feeding and infections (1000 L3 3 times a week for 3 weeks) started. (B) Infections stopped. (C) SL hay feeding switched to BG hay feeding. Asterisk (*) indicates significant ($p < 0.05$) difference based on log-transformed data.

3.2.2. Blood packed cell volume

Mean PCV for both groups were similar ($p > 0.05$) throughout the study and decreased from around 36% to 25% over time.

3.2.3. Worm count

The only worms found were *H. contortus* in the abomasum. None were found in the small or large intestine. Mean *H. contortus* count in the SL group (425) was numerically lower than the BG group (575), but not different ($p > 0.05$). The female to male ratio was higher for the SL group (1.43) than the BG group (1.23), but the difference was not significant ($p > 0.05$).

4. Discussion

Under the conditions of these studies, SL was effective in reducing FEC in natural infections and suppressing FEC in newly acquired experimental infections in lambs. This effect is likely related to both a direct effect on the adult worms and reduced fecundity of female worms. The 67.2% reduction in worm burden in Study 1 most likely accounted for a substantial part of the lower FEC. Once the effect of SL was removed (Day 49), FEC increased notably which is an indication that there was also an effect on fecundity. A fecundity effect appeared to be more pronounced in Study 2 where the percent reduction in FEC during SL feeding was similar to Study 1, but the reduction in worm burden was much less (26.1%). The observation that FEC started to increase before SL feeding was stopped in Study 2 can be explained by one animal whose FEC increased substantially from Day 42 to Day 49. That particular animal may have voluntarily gone off feed for some undetermined reason. The small reduction in worm

burden in Study 2 indicated that SL probably did not have much of an effect on establishment of maturing worms. Therefore, if establishing worms in Study 1 were not affected much, this may have accounted for the majority of the worms in the SL group and the effect on established worms may have been greater than what was observed. This effect on established worm burden could be due to direct toxic effects on the worms or changes in the abomasal environment that favor worm expulsion. In a study with goats fed SL hay, Shaik et al. (2006) suggested direct toxic effects as the likely cause for substantially reduced numbers of *H. contortus*, *T. circumcincta*, and *T. colubriformis* in goats fed SL compared to BG hay. In the current study, a sudden change in the abomasal environment due to the introduction of CT may explain the greater reduction effect on mature (established) compared with immature (establishing) worms. Worms entering the already changed environment experience no such sudden shift and therefore may not be affected. Differences in PCV reflect the relative infection level between SL and BG groups for both trials.

These findings support those reported in goats grazing SL (Min et al., 2004) and in goats experimentally infected and fed SL hay (Shaik et al., 2004, 2006). Min et al. (2004) demonstrated an effect on worm fecundity by switching grazing goats from SL to ryegrass pasture. On SL pasture, FEC decreased and when switched there was an immediate increase in FEC which can only be explained as an effect on fecundity. Shaik et al. (2006) reported significant reduction in numbers of *H. contortus*, *T. circumcincta* and *T. colubriformis* in goats fed SL hay compared with BG hay. It was also reported that the effect was greater on female than male worms for each species, which was in contrast with the results of this study where there was no obvious difference in female and male worms for any group, and proportionately more females were actually found in the SL groups. These results also support the findings of Niezen et al. (1998), where FEC and worm burdens (primarily *T. colubriformis* and *T. circumcincta*) were reduced in sheep that grazed sulla, a CT containing forage. However, these results seem to be in contrast to those reported by Athanasiadou et al. (2001) in sheep given CT containing quebracho extract. The quebracho extract had an effect on the small intestinal worms *T. colubriformis* and *N. battus*, but not on the abomasal worms *H. contortus* and *T. circumcincta*. However, it was noted that the short exposure (3 days) to the quebracho extract may not have been long enough for an effect on the abomasal worms. The decrease in *H. contortus* FEC in this study (1 week after the start of SL

feeding) was much quicker than that observed for *T. colubriformis* by Athanasiadou et al. (2000), where the decrease occurred over 6 weeks. Again, the species of worms involved were different which could account for the difference. Athanasiadou et al. (2000, 2001) speculated that differences may be indicative of an effect due to the different tannins involved and/or the different species of worms. Further, that the particular chemical structure of CT could account for different effects on animal physiology and digestion (Mueller-Harvey and McAllan, 1992). The quick FEC decrease seen in this study indicates that SL may have greater practical application potential in treating animals infected by *H. contortus* and minimizing pasture contamination than quebracho extract.

Paolini et al. (2003c) reported that the effects of quebracho extract varied with the stage of *T. colubriformis* and *T. circumcincta* in goats. It was indicated that the effect on established adult worms was limited to reduced fecundity, while establishment of incoming L3 was reduced. In another study, also in goats, it was reported that quebracho extract reduced fecundity but not the burden of established *H. contortus* infection (Paolini et al., 2003b). This is in contrast to the results of the studies reported here, which showed a possible lethal effect on established worm burden in sheep. In another study, Paolini et al. (2005b) reported that quebracho extract and sainfoin hay did not affect establishment of incoming L3 of *H. contortus* in goats, which is supported by the results of the current study. As mentioned above, this could be attributed either to differences between worm species or between tannin structures, but a difference in animal species (goat/sheep) may be involved.

In addition to its potential anthelmintic activity, SL could be considered as a viable forage alternative. It flourishes on poor, eroded, and infertile soils, is drought, insect and disease resistant, and produces high forage yields. SL is also considered an excellent soil builder as it drops a large quantity of leaves that improve soil quality (Kalburtji et al., 1999). However, SL is considered a noxious weed in some parts of the southeastern US and livestock (mainly cattle) may resist grazing it due to the high CT concentration (Ohlenbusch et al., 2001). This may hinder using SL as a forage. However, goats will readily graze SL (Min et al., 2004) and sheep and goats will consume SL as hay (Terrill et al., 1989; Shaik et al., 2006). The process of drying has been shown to cause oxidative changes in tannins (Goldstein and Swain, 1963) and may be a very practical method of improving palatability, as well as eliminating many of the other problems associated with

treatment by fresh forage. Since effects of CT are tied to their concentration in the forage, which varies as the plant matures, it is also nearly impossible to ensure that the optimal concentration of CT is present during the peak infection season when it would be needed (Paolini et al., 2003b). Uncertainty related to weather and other factors could also impair ability to produce the forage at the needed time. Converting the plant to hay when it reaches its optimal growth stage enables it to be stored and available when needed. In addition, hay can be further processed into ground meal, pellets and/or cubes for incorporating into various feeding programs. It may also be possible to process fresh SL forage or hay to obtain extracts that have anthelmintic properties.

5. Conclusions

SL hay was shown to be effective at reducing FEC in sheep, and therefore may prove to be a useful practical tool for reducing pasture contamination by *H. contortus*. It also appeared to be useful for eliminating established adult worm burdens similar to or better than what an anthelmintic treatment might achieve when resistance is present. Further studies to assess SL efficacy against worms other than *H. contortus* in sheep will also help to define its application potential.

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