# Short Term Effect of *sericea lespedeza* on Strongyle Fecal Egg Counts in Mature Horses By Marrissa J. Blackwell, Anthony Britt, Madison Fagan, Kari K.

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### Abstract

Control of parasitic infections in the equine industry is primarily accomplished through deworming programs that implement the use of anthelmintics, with the primary target in mature horses being small strongyles. Due to widespread use of common anthelmintics, the overall effectiveness of these treatment plans is at risk of diminishing. Though there have not been significant breakthroughs in equine anthelmintic resistance, small ruminant research has focused on non-chemical control for gastrointestinal nematodes that involves feeding plants containing condensed tannins (CT), namely sericea lespedeza (SL), to combat parasite populations. While no such studies have been conducted on equine subjects, SL may have potential as an alternative treatment. The objective of this study was to analyze the effects of SL as compared to russell bermudagrass (RB) with regards to parasite load in horses, as well as assess palatability and CT content of SL. Sixteen horses were divided into two treatment groups, each receiving 1.5% of body weight in either SL or RB hay daily over a four week period. Fecal egg counts (FEC) performed by mini-FLOTAC technique were conducted at d 0, 14, and 28 of the feeding trial as well as on d42 (two weeks after removing from diets). Average initial (d0) FEC for horses on both treatment groups was high and not statistically different (SL=393±49, RB=493±51, P=0.98). All horses showed significant increases in FEC by d42 (P<0.001), with no differences between treatment groups (P>0.05). However, the overall binding CT content of SL used in this study was considerably lower than values reported in previous small ruminant research. In regards to palatability, horses on the SL diet had significantly lower daily refusals, suggesting that SL was more palatable than RB. While no effect on parasite load was observed in this study, future studies could incorporate longer feeding times, feeding forages with higher CT content, and the combination of feeding SL in conjunction with a deworming program.

### Introduction

Control of parasitic infections in the equine industry is primarily accomplished through deworming programs that implement the use of anthelmintics. In the 1960s, rotational deworming practices gained popularity as a significant reduction in the most prevalent parasite, large stronglyes (Strongylus vulgaris), was noted. Due to the widespread use of anthelmintics (dewormers), the large strongyle population was virtually eradicated. Today, the most predominant parasitic threat to horses is small stronglyes (cyathostomins).

Cyathostomins have a unique life cycle. Eggs are passed from adult worms through the feces to begin development in pasture. Larval stages 1-3 take place here with the rate of development highly dependent on climate. Once the infective L3 stage is reached, the larvae become encased in a protective membrane equipping them with the ability to withstand freezing temperatures and allowing them to remain on pasture for longer periods of time. The larvae are ingested by the horse, allowing for the removal of the protective sheath as they enter the mucosa of the large intestine. Unlike large strongyles, small strongyles have the unique ability to encyst themselves in the gut wall until conditions

are favorable for their survival. The L3 larvae can remain encysted for up to 2 years. Once they emerge, larvae continue developing to the L4 and L5 stages, eventually reaching maturity as an adult parasite in the cecum or colon. At this stage they lay eggs to be passed through the feces as the next generation of cyathostomins begin.<sup>1</sup> This distinctive trait of being able to encyst in the intestine has led to the development of small strongyle resistance to rotational deworming, as most anthelmintics are only able to kill the parasites in the lumen of the intestine. In response to the increasing prevalence of anthelmintic resistance, the AAEP (American Association of Equine Practitioners) released a new set of recommendations that base parasite control on individual fecal egg counts rather than broadly deworming all horses at regular intervals.<sup>2</sup> However, the industry's practices are more prominently based in tradition and have lagged in adopting this evidence-based approach.3

Due to the widespread use of anthelmintics at high frequencies, as well as a lack of new methods, the overall effectiveness of conventional anthelmintics is at risk of diminishing.<sup>1,3</sup> Anthelmintic resistance is an inherited trait that can be passed from one generation of parasite to the next. The great population size in conjunction with high levels of genetic diversity has allowed small stronglyes to rapidly develop resistance to anthelmintics. Currently, there are three main classes of anthelmintics: benzimidazoles (fenbendazole, oxibendazole), tetrahydropyrimidines (pyrantel), praziquantel and macrocyclic lactones (ivermectin, moxidectin). Possibly due to poor deworming practices, resistance to these anthelmintics has been reported worldwide.<sup>4</sup> The World Association for the Advancement of Veterinary Parasitology considers anthelmintic resistance to occur if there is a 95% or less effective rate of the drug to the target parasite load.<sup>5</sup> It has been shown that the percentage of farms in the southern United States found to harbor resistant small strongyles was 97.7% for fenbendazole, 53.5% for oxibendazole, and 40.5% for pyrantel pamoate. In this study, 0% of farms harbored small strongyles that were resistant to ivermectin, however, it has been recently shown that resistance to ivermectin is common in another equine parasite, ascarids.<sup>3</sup> Substantial anthelmintic resistance has been noted among other livestock species as well, particularly small ruminants. The first reported case of anthelmintic resistance in the United States dates back to the late 1950s when producers discovered their sheep were showing resistance to phenothiazine.<sup>6</sup> Soon thereafter, reports followed exposing resistance to benzimidazoles, a class of anthelmintics still used today.<sup>7</sup> In the past 50+ years, as new anthelmintics have been produced, parasite resistance has eventually followed, leading researchers to look to new mechanisms to combat their ever growing problem.

Though there has not been a significant breakthrough in equine anthelmintic treatment, small ruminant research has recently focused on non-chemical control methods for gastrointestinal nematodes (GIN) that involve feeding plants containing condensed tannins to combat populations such as haemonchus contortus.<sup>8,9</sup> Many organizations such as the American Consortium for Small Animal Ruminant Parasite Control have sought to further study the potential of these plants, more specifically sericea lespedeza (SL). Many research studies have shown that SL is effective in reducing parasite counts in small ruminants, though the exact mechanism by which this occurs has still not been confirmed.

Studies on the effectiveness of SL on parasite populations in small ruminants have shown promising results. Terrill et al. (2009) showed a reduction in FEC of goats fed a diet of grain and either 25%, 50% or 75% SL as compared to bermudagrass, with a greater reduction in FEC seen with higher percentages of SL fed.<sup>10</sup> Lange et al. (2006) reported lambs fed SL hay for 7 weeks saw a 67-98% reduction in FEC as well as adult worms as compared to counterparts fed a bermudagrass diet.<sup>11</sup> Further demonstrating the efficacy of SL, Min et al. (2005) found Angora does grazing SL for 81 days resulted in a reduction of FEC, adult worms, and larval activity.<sup>9</sup>

Due to the fact that tannins were developed as a defense mechanism for plants, the first known variety of SL was extremely high in tannin content and therefore had extremely low palatability. Because of this, the original SL introduced to the United States in response to drought was not readily grazed by livestock, and lespedeza varieties remained unpopular as a livestock forage sources for many years. Over time, lespedeza has become a viable forage source, with several varieties being developed that contain overall lower tannin content, fine stems, higher protein content, and in some varieties, higher digestibility.12 Though low tannin content improves palatability, higher tannin content has been correlated with lower fecal egg counts in small ruminants. SL has historically been fed to horses, with many current nutritional websites mentioning its virtues, namely increased protein and calcium levels and acceptable digestibility when harvested properly. While not currently widespread in popularity, it is evident that SL has been, and is still, fed safely to horses, though no research is readily available examining the anthelmintic effects of CT in the equine diet. The aim of this study was to assess the effects of short term (4 week) feeding of SL on existing strongyle populations in adult horses as well as assess palatability and CT content of SL as a forage source for horses. Small strongyles were analyzed for this study as they are the priority target in deworming protocols and are closely related to the haemonchus contortus species that small ruminant research has previously focused on.

### Methods

### 2.1 Treatment Groups and Diet

All protocols were approved by the UGA Institutional Animal Care and Use Eighteen stock-type horses Committee. (11.7±3.3 yrs, 552±49 kg) were used in a 4 week feeding trial to study the effects of daily feeding of SL hay as compared to Russell bermudagrass (RB) hay on existing parasite load. Horses had previously been group housed in large (12+ hectacre) pastures and had not received anthelmintic treatment for a minimum of 4 months prior to initiation of the study. FEC were performed on all horses as described below on d0, and horses were then randomly assigned to one of two groups (SL or RB). Body weight (BW) of all horses was recorded by electronic scale on d0, and forage amounts were assigned based on this weight. Beginning on d0, 9 horses were randomly placed on a diet of 1.5% BW in SL hay in addition to pasture turnout, and 9 horses began receiving a control diet of 1.5% BW in RB hay in addition to pasture turn out. Amount of hay fed is based on recommendations for equine dry matter intake. Horses were housed in stalls for approximately 6 hr/d to allow for individual consumption of hay. For the remainder of the day, horses were housed in one of two adjacent small pastures (2.8 hectacre) for turnout, with each pasture having an equal number of RB and SL horses. Hay was weighed using an electronic scale each day, and any hay left uneaten at the end of the six-hour period was weighed and recorded. 2.2 Fecal Collection Protocol

Individual fecal samples were collected on two consecutive days via rectal palpation at the following time points:

-Prior to horses beginning forage diets (Day 0)

-Two weeks into feeding trial (Day 14)

- Last day of feeding trial (Day 28)

-Two weeks after end of trial (Day 42) All samples were stored at 35°F, and samples were analyzed within one week of storage to ensure viability.

### 2.3 Fecal Analysis

Two subsamples from each FEC collection time point were analyzed, giving a total of 4 subsamples for each time point (i.e., 2 subsamples from 2 consecutive days for D0, etc.). Due to the proven accuracy of this method, fecal analysis was performed using the mini-FLOTAC procedure as described by Barda et al., 2014.13 Briefly, each subsample was created by measuring 5 grams of fecal sample with a Mettler-Toledo Bd202 weigh scale (Mettler-Toledo LLC., Columbus, Ohio). The fill-FLOTAC container (Naples, Italy) was filled with 45mL of Feca-Med sodium nitrate solution with a specific gravity of 1.200 (Vedco Inc., Missouri). The conical collector on the lid of the fill-FLOTAC container was filled with the subsample and the surface was leveled. The lid with the subsample was screwed on the fill-FLOTAC container tightly. The subsample was agitated by lifting the plunger



Figure 1. Mean fecal egg count over time for horses on either a lespedeza or bermudagrass hay diet. Lespedeza: Horses were individually fed 1.5% of body weight in sericea lespedeza hay daily. Bermudagrass: Horses were individually fed 1.5% of body weight in Russell bermudagrass hay daily.

*Time: Represents number of days on hay diets. Overall treatment effect: P=0.14* 

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on the lid up and down while twisting until the subsample was completely homogenized with the sodium nitrate solution. The mini-FLOTAC slide's three parts were assembled by following manufacturer's instructions. The chambers were filled with the homogenized sample from the fill-FLOTAC with the pipette attached to the lid while holding the mini-FLOTAC assembly at an angle. The sample was then allowed to sit and rest for 10 minutes to allow the eggs to rise and adhere to the reading disc grids. Slides were then read using the 10X objective lens of a Labomed binocular microscope (Labomed Inc., California). The fecal egg count was obtained using the equation below:

(# of eggs in Grid 1 + # of eggs in Grid 2) X 5 = Fecal Egg Count

2.4 Statistical analysis

Four replicates of FEC were generated for each time point and incorporated into the statistical analysis. Time points were statistically analyzed with SAS version 9.4 (Cary, NC, USA) using proc mixed for repeated measures over time. Time, treatment and treatment by time interactions were analyzed for each parameter, with a post hoc PDIFF comparison used to compare treatment means within each time point. Data with P<0.05 were considered statistically significant.

2.5 Assessment of condensed tannin levels in hay samples

Hay samples were collected from at least 15 bales of each hay using a standard hay corer. Proximate analysis of hay samples was performed, and condensed tannin levels were analyzed using two different techniques: 2.5a Method 1: Extractable procyanidin analysis

Proximate analysis was performed and extractable procyanidins quantified via the method adapted from Payne et al. 2010 and described below:<sup>14</sup>

Extraction of lipids from hay samples

A Soxhlet apparatus was employed to extract the lipids from the two types of hay. A representative sample of ground hay was weighed into a Whatman cellulose extraction thimble (43 mm *i.d.* × 123 mm *e.l.*, VWR International, Suwanee, GA, USA), and the mass recorded. Glass wool was placed in the mouth of the thimble to ensure that the contents would remain in place during extraction, which was performed using ~350 mL of hexanes for 20<sup>th</sup>. Upon completion, the thimble was removed from the Soxhlet extraction tube and the contents were airdried overnight. Hexanes were removed from the lipid extract via a Büchi Rotavapor R-210 using a V-700 vacuum pump connected to a V-850 vacuum controller (Büchi Corporation, New Castle, DE, USA) at 45°C. The crude lipid portion was weighed for gravimetric analysis.

Extraction of phenolic compounds

Each defatted hay sample was removed from the air-dried thimble and placed in a 500mL Erlenmeyer flask. A 100-mL portion of extractant ((CH<sub>3</sub>), CO/H<sub>2</sub>O/CH<sub>3</sub>COOH solvent mixture, 70:29.5:0.5 v/v/v) at a ratio of ~6:10 defatted hay:extraction solvent (w/v) was used to extract the phenolic compounds. Briefly, the contents in the flasks were heated at 50 °C for 30 min in an orbital-shaking water bath (New Brunswick Scientific, New Brunswick, NJ, USA). The extraction was performed  $3\times$ , the supernatants pooled and acetone removed by the Rotavapor. The aqueous portion was poured into crystallization dishes (100 × 50 mm, dia. × H), covered with filter paper, and placed in a -80 °C freezer until completely frozen. The samples were then lyophilized (Labconco Freezone 2.5 L freeze dryer, Labconco Corp., Kansas City, MO, USA). The dried extract was weighed, placed in amber-colored vials, capped and stored at 4 °C until analyzed. The lipid and phenolic extractions were completed in triplicate for each hay sample. *DMAC assay* 

The total procyanidins content in the acetonic crude extracts were quantitated by the 4-(dimethylamino) cinnamaldehyde (DMAC) assay according to Payne et al. (2010).<sup>14</sup> Briefly, 50 µL of methanol and 50 µL of standard solutions (+)-catechin or 50 µL of hay crude phenolic extracts, dissolved in methanol, were respectively added to a COSTAR® 96-well clear, non-sterile, nontreated microtiter assay plate and then mixed with 250 µL of the DMAC solution. This reagent was prepared fresh each day by dissolving 30 mg of DMAC in 30 mL of 1:9  $(\nu/\nu)$  HCl and reagent alcohol. Absorbance readings were recorded with a FLUOstar Omega microplate reader (BMG LABTECH Inc., Čary, NČ). Assay conditions comprised bottom scanning every 1 min over 12 min at  $\lambda = 640$  nm at an incubation temperature of 25 °C. The plate was shaken for 3 s before





Figure 2. Change in mean fecal egg count over time for horses on either a lespedeza or bermudagrass hay diet. Horses remained on hay diets from D0 to D28.

Lespedeza: Horses were individually fed 1.5% of body weight in sericea lespedeza hay daily

Bermudagrass: Horses were individually fed 1.5% of body weight in Russell bermudagrass hay daily



Lespedeza: Horses were individually fed 1.5% of body weight in sericea lespedeza hay daily

Bermudagrass: Horses were individually fed 1.5% of body weight in Russell bermudagrass hay daily

\* indicates difference in treatment groups at specific time point (P<0.01) Overall treatment effect: P<0.0001

Overall treatment effect: P=0.50



Figure 4. Condensed tannin analysis of sericea lespedeza hay and Russell bermudagrass hay diets. Figure 4a (left) represents the extractable procyanidin levels of hay fed to horses in this study. Figure 4b (right) represents the protein precipitable condensed tannin levels from hay collected the subsequent year from the same fields and harvest time.

measurement. The maximum absorbance during 12-min readings was used for calculation. Total procyanidins contents were expressed as mg (+)-catechin equivalents/g hay sample (f.w.). All assays were performed in triplicate.

## 2.5b Method 2: Determination of protein precipitable CT

Additionally, RB and SL hay samples from the subsequent year's cutting of the same harvest time and same fields were analyzed using a different technique to measure both total phenolic content as well as levels of protein precipitable condensed tannins (biologically active tannins). Samples from each type of hay were sent to Texas A&M Aglife Research, and protein precipitable CT were analyzed using methods described by Naumann, et al. 2014.<sup>15</sup>

### Results

There were no statistical differences noted between treatment groups at any time point during the feeding trial. Both treatment groups at Day 0 show relatively high parasite counts (SL=393±49, RB=493±51 epg). By comparison, at D28 of the study, a decrease was seen in parasite count for both treatment groups (SL=123±70, RB=116±44 epg). At D42 (two-weeks post-trial) the FECs for both treatment groups was significantly higher (SL=986±124, RB=1262±119 epg) than time points between D0 and D28 (Figure 1). Changes in FECs over time were recorded in order to further examine changes in parasite populations relative to individual initial FEC, with no differences noted between treatment groups at any time point (Figure 2).

Weigh back data for the first 3 weeks of

the study is summarized in Figure 3. Over the course of the first three weeks, horses receiving SL diets had less hay refused as compared to RB horses (P<0.0001). By week 4, horses consumed most of the hay on both diets, and weigh backs were minimal and not recorded. Proximate analysis showed that SL hay had comparable moisture levels to RB, was somewhat higher in carbohydrates and lipids (SL= 70.23, 2.06%; RB= 65.92, 1.62% respectively) and lower in protein (SL=11.67%, RB=16.93%) (Table 1). CT content of SL hay was higher than that of RB measured both by extractable procyanidin levels (Figure 4a) as well as by precipitating CT levels from subsequent year's hay samples (Fig 4b).

### Discussion

The aim of this study was to assess the nutraceutical value of SL as compared to RB in regards to parasite load in horses and to compare these results to studies done in small ruminants fed SL. The results of our trial demonstrate that feeding 1.5% BW in SL over a short time period of 4 wks had no effect on reducing existing FEC or in preventing an increase in parasite populations in horses. Our data show that at the beginning of this feeding trial, both experimental groups had relatively high parasite counts. By D42 these numbers had drastically increased beyond the original levels. This data reflect the normal 30-day life cycle of small strongyles that occurs under favorable conditions where the horse ingests parasite larvae from infective pastures that then develop in the intestines and lay eggs to be passed again. Given the higher than recommended stocking rate in this trial (<0.4 hectacre/animal), by D42 we would expect to see an increase in parasite loads as compared to when horses started the project and had been housed in larger pastures. The increase in FEC seen in both groups implies that feeding SL for a short time frame did not beneficially affect parasite load in mature horses. These findings are contradictory to the results of several previously cited studies in small ruminants. A study conducted by Lange et al (2006) examined the effects of SL on parasite load in lambs when fed as a hay for 49 days and then removed for 14 days.<sup>11</sup> In this study, feeding SL ad libitum reduced worm burden by an anywhere from 67-98% as early as 7 days after initiation of the diets. Removal from SL for 2 weeks post-feeding trial resulted in an increase in FEC, but SL fed lambs still had significantly lower FEC as compared to those fed bermudagrass. By contrast, horses in the current study showed no decrease, and in fact a dramatic increase, in FEC while on SL diets.

A potential explanation for the lack of anthelmintic effect of SL in this study may be the levels of CT found in the forage when analyzed. Both RB and SL samples for our study were analyzed for CT content using two different methods. In the first method, CT levels were assessed by analyzing the procyanidin content of both hays. While SL hay did have a higher procyanidin content as compared to that of RB hay (1.94 mg/g vs 0.02 mg/g), it was considerably lower than CT levels reported for SL in other small ruminant studies (0.194% as compared to levels of 5% or higher).<sup>16</sup> However, Mechineni et. al (2014) reported that the majority (>90%) of CT in SL forage are of the prodelphinidin type rather than procyanidin.<sup>17</sup> Ådditionally, the first method used analyzed only extractable CT rather

%	Russell Bermudagrass	Sericea Lespedeza
Moisture	9.76	9.90
Crude Lipids	1.62	2.06
Crude Protein	16.93	11.67
Carbohydrate	65.92	70.23
Ash	5.77	6.13

Table 1. Proximate analysis for Russell bermudagrass and sericea lespedeza hays fed to horses for duration of study.

than also accounting for protein bound CT. To examine this further, samples from the subsequent year's hay crops of the same month and field were analyzed for protein precipitable condensed tannins which are generally considered to be biologically active. Again, the levels of CT in SL (13.4 mg/g, 1.34%) were higher than that of RB (0 mg/g), but considerably lower than what was reported by Mechineni et. al in 2014 (125 mg/g, 12.5%).<sup>17</sup> It is well understood that high levels of CT result in reduced palatability, which may explain why SL in our study was so readily consumed by horses. However, future studies may want to investigate how prodelphinidin and procyanidin types produce anthelmintic effects as well as explore feeding varieties of SL containing levels of CT more closely resembling that which has been fed to small ruminants.

Despite the vast literature available regarding tannins and their effect on the small ruminant gastrointestinal parasite populations, little data exists in regard to anthelmintic effects of SL in other species of livestock. Our study offers viable preliminary data to act as a basis for future studies. Though no significant effects on strongyle populations were noted, our data provided insight on alternative methods for future studies as well as justification to suggest that SL is a palatable forage that could potentially act as a highly nutritive roughage for horses.

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